THE EFFECTS OF INSPIRED POLLUTANTS ON RESPIRATORY AND CARDIOVASCULAR FUNCTION IN HEALTHY YOUNG ADULTS

A Thesis
by
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Submitted to the School of Graduate Studies
at Appalachian State University
in partial fulfillment of the requirements for the degree of
MASTER OF SCIENCE

May 2021
Department of Health & Exercise Science

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Abstract

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Inspired pollutants have been associated with an increased risk of developing respiratory and cardiovascular diseases. While epidemiological studies have attempted to elucidate potential correlations, few have been able to determine true impacts of the pollutants much less elucidate a mechanism of action. The purpose of this thesis is to disseminate the knowledge of previously collected data pertaining to inspired pollutants including: the effects of formaldehyde (FA) on pulmonary function, the effects of FA on vascular function, and the effect of electronic cigarettes on metabolic and ventilatory measurements during exercise. As an added measure to the FA manuscripts, biomarkers of oxidative stress and inflammation were assess immediately prior to and following an acute exposure to FA which has potential to reveal a mechanism. While FA influence the vasculature, pulmonary function remained unchanged as a percent difference from pre-FA exposure. Additionally, no physiological differences were observed in males comparing a placebo incremental exercise test to an experimental electronic cigarette incremental exercise test. In essence, inspired pollutants may damage otherwise young and healthy individuals; however, their body is capable to compensate for the onslaught of inspiratory damage.

Dedication

This dedication is to my parents, Kelly and Scott Augenreich, and to their spouses. They have provided the financial and emotional support in this insane endeavor that I call a career, to which I could not endure on my own. Additionally, I dedicate this to the promise of a career supported by my little family including Nathan Wonsch, and our silly pets Milla and Charlotte.

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Foreword

The content of this thesis is intended to be submitted for publication in two different journals. The first manuscript, entitled "Vascular Dysfunction and Oxidative Stress Caused by Acute Formaldehyde Exposure in Female Adults," has been accepted to the *American Journal of Physiology: Heart and Circulatory Physiology*. The second manuscript, entitled "Pulmonary Function Following Acute Formaldehyde Exposure in Young Adults," is written to be submitted to the *Journal of Environmental Toxicology*. The third manuscript, entitled "Metabolic and Ventilatory Responses to Exercise Following Electronic Cigarette Usage," is intended to be submitted to the *Journal of Environmental Toxicology*. All manuscripts have been formatted according to their respective journal regulations. Additionally, all written work aiding to the background of this thesis has been formatted to the *American Journal of Applied Physiology: Heart and Circulatory Physiology*. The background information for this document will have references found at the end of the document; however, each manuscript will maintain its own reference section within its respective chapter.

Chapter 1

The contents of this thesis document are composed to satisfy requirements set forth. The committee have agreed that the required materials are three manuscripts with a central topic based on environmental pollutants and how they may influence respiratory or cardiovascular physiology. Given the deviation from a typical thesis format, the format of this document is intended to display the topics as individual manuscripts with their respective measures and pollutants, while keeping the formaldehyde background and manuscripts together in Chapter 2 and Chapter 3 comprising electronic cigarette background and manuscript. The first manuscript of Chapter 2 is entitled Vascular Dysfunction and Oxidative Stress Caused by Acute Formaldehyde Exposure in Female Adults which measures the effect formaldehyde has on the vasculature as well as compared pre- and post- exposure biomarkers of oxidative stress to attempt to elucidate a mechanism. Second is a manuscript entitled Pulmonary Function Following Acute Formaldehyde Exposure in Young Adults which assesses the effect of formaldehyde on pulmonary function. The two manuscripts of Chapter 2 I had part in composing the study design, data collection and organization, statistical analysis, manuscript composition and creation of figures. Finally, the third manuscript entitled Metabolic and Ventilatory Responses to Exercise Following Electronic Cigarette Usage exposes subjects to electronic cigarette vapor, or placebo, to assess if responses to exercise are diminished or amplified. The manuscript of Chapter 3 I had the opportunity to process data, statistically analyze said data, compose a manuscript and creation of figures.

These three manuscripts are centered on the concept that an inspirate, both naturally occurring formaldehyde and synthesized electronic cigarette vapor, have the potential to influence tissues of the respiratory system. In the event that the environmental pollutants (EP) are small enough, there is

potential for the inspirate to cross the alveolar membrane into the blood stream where vasculature can be influenced. These manuscripts combine respiratory characteristics with cardiovascular physiology to disseminate the integrative nature of these systems.

The study of human physiology has long been of interest so much so that prediction equations have been established to determine when individuals deviate from expected values, potentially pointing to a physiological impairment. For example, Wasserman developed equations to determine the minute volume of consumed oxygen against no resistance as well as at peak exercise in humans on a cycle ergometer (1) and is the foundation for articles that look at deviations from expected in clinical populations (2).

When observing metabolic parameters of rest, regression equations have been established by observing heat production (3):

$$\text{Male: } h = 66.4730 + 13.7516\ (kg) + 5.0033\ (cm) - 6.7550\ (yr)$$

$$\text{Female: } h = 655.0955 + 9.5634\ (kg) + 1.8496\ (cm) - 4.6756\ (yr)$$

with knowledge that sex, height, weight and age having an effect on resting metabolic rate, there must be acknowledgement that factors beyond human control influence metabolic rate including oxidative stress from hydrogen peroxide and superoxide (4). Furthermore, the same principles can be extrapolated to exercise. Again, the Wasserman equations are intended to predict $\dot{V}O_{2max}$ of otherwise healthy individuals (2):

$$\text{Male: } \dot{V}O_2\ (mL \cdot min^{-1}) = W\ [\ 50.75 - 0.372(A)]$$

$$\text{Female: } \dot{V}O_2\ (mL \cdot min^{-1}) = (W - 43)\ [22.78 - 0.17(A)]$$

where W is mass in kilograms and A is age in years. It should be noted that updated equations have been published by Wasserman and colleagues in 1999. Given this information, it can be assumed that oxidative stress incurred from EP will eventually lead to increases in metabolic rate thus altering the normal homeostatic conditions of systems.

In healthy individuals, measurements from an incremental exercise test such as the proportional increases of heart rate and $\dot{V}O_2$ (5) can elucidate the physiological principles at the recorded time frame. Ventilation ($\dot{V}_E$) has two inflections to the trend line in which correlate to the aerobic threshold and the anaerobic threshold as work rate (WR) increases towards subject $\dot{V}O_{2max}$ (6, 7). The second inflection of the ventilation line where $\dot{V}_E$ increases disproportionately to WR is labeled the anaerobic threshold and is hyperventilation relative to oxygen consumption (6, 8, 9).

When observing the metabolic responses to an incremental exercise test, specific variables are assessed for their likeness to normal trends, for instance resting respiratory exchange ratio (RER) is expected to be approximately 0.70 as lipids are the primary metabolite at rest as aerobic metabolism has the largest adenosine triphosphate (ATP) production per energy investment (10). The RER is the minute volume of produced carbon dioxide ($\dot{V}CO_2$) and the minute volume consumed oxygen ($\dot{V}O_2$); due to the beta oxidation of fatty acids, most commonly palmitoyl-stearoyl-oleoyl-glycerol in human adipose tissue (11), to acetyl-CoA, and further reactions via the Krebs Cycle, the overall combustion reaction results in a $\dot{V}CO_2$ = 1.427 L and $\dot{V}O_2$ = 2.019 L, thus an RER = 0.707. Additionally, there is a shift in metabolism from lipid oxidation to greater carbohydrate metabolism which is anaerobic during glycolysis (10) and has a lower oxygen consumption during oxidations (0.746 mL·g-1) and equivalent production of carbon dioxide (0.746 mL·g-1) thus an RER value of 1.00 (11). In a system similar to Simonson & DeFronzo (1990), *in vitro* experiments would reach a ceiling value and is described as the respiratory quotient (RQ = 1.00); however, *in vivo* experiments measure gas volumes at the mouth and is influenced by the dynamics of the respiratory system with maintaining homeostasis. At high work rates, such as those in an incremental exercise test, the respiratory system is responsible for maintaining blood pH by hyperventilating to buffer for the increasing proton concentration by freeing bicarbonate (12). To summarize, *in vivo* experiments measure the $\dot{V}CO_2$ produced and the $\dot{V}O2$ consumed and express the value as a ratio (RER) which can be greater than 1.00 due to buffering via respiratory system.

The breathing mechanics of normal exercise describes the pressures generated, the flow and volume of air. The assessment of breathing mechanics requires that lung volumes and spirometry are measured as these values complete the picture when assessing for variables such as expiratory flow limitation. A normal response to exercise is for the end-inspiratory lung volume (EILV) to increase from rest with each increase of WR (13) whereas end-expiratory lung volume (EELV) decreases slightly from resting EELV (13). These changes in volumes can be integrated to a single volume, tidal volume (V_T), though this is limited as the inspiratory and expiratory reserves are not accounted for in V_T. Additionally, the duration of inspiration and expiration (T_i and T_e, respectively) are observed as a function of flow and made relative to the total duration of a breath (T_{tot}) (14). Durations of breaths are expected to have a longer duration during expiration as the act of expiration is passive, until high breathing frequency (f_B) is required; conversely, inspiration requires contraction of the diaphragm and additional trunk muscles at higher V_T. Therefore, relative to T_{tot}, T_i tends to be <0.50 and T_e >0.50 both at rest and moderate $\dot{V}_E$ (15). Furthermore, increasing WR causes the T_i/T_{tot} and T_e/T_{tot} to converge closer to 0.50 suggesting that T_i/T_e are equivalent (15).

A purpose for the measurement of breathing mechanics is expiratory flow limitation (EFL) (16). When overlaying a tidal breath on a maximal flow volume loop (FVL), an EFL can be observed as an overlap of the tidal and maximal FVL (tFVL and mFVL, respectively) (17). However, EFL can be described by transpulmonary pressures as certain pressures at given volumes did not increase flow (16).

Much of cardiopulmonary function is made relative to the subject, one set of variables being the pulmonary function (2). As suggested by Johnson *et al.* (17), $\dot{V}_E$ relative to maximum voluntary ventilation (MVV) is lesser of a predictor of ventilatory constraints than that of EFL. Additionally, the mFVL is used from measures of spirometry in the assessment of EFL (17). The mFVL along with lung volume assessments, via body plethysmography observing Boyles Law or nitrogen/helium washouts (18), are essential in describing operational lung volumes and assessing the breathing mechanics during rest and exercise (19).

As for vascular assessments, flow-mediated dilation (FMD) has been established as a measurement of vascular health in large part due to the relationship between brachial artery dilation and coronary dilation capabilities (20) as well as indices for atherosclerotic events (21). The rationale behind FMD is that, with brachial artery having a relationship with coronary arteries, shear stress induces nitric oxide (NO) release (22) thus dilating vessels. In healthy persons, FMD is expected to be a higher percentage (7-18 %) than those of smokers (-1-17 %) and coronary artery disease individuals (-6-7 %) (21) which suggests that individuals that have been exposed to higher oxidative stress, such as smoking (23), may have impaired vascular function.

Additionally, the measure of reactive hyperemia is assessed simultaneously with FMD measures and aids to measure vascular resistance (24). This further plays off the principle that NO is essential to endothelial function as it reduces resistance by increasing diameter (21).

The variables concerning exercise metabolism, ventilatory dynamics, and vascular function, are what can be expected from otherwise healthy adults; however, EP can play a crucial role in the regulation of these variables.

Human physiology is a highly integrative topic that spans beyond that of just internal systems. Basic knowledge suggests that the environmental conditions in which organisms reside will influence that organism; however, to what extent, the mechanisms of action, and the likelihood of exposure to certain stimuli tends to be a far more intricate topic of discussion. Studies have gone as far to suggest a correlation of environmental pollution (EP) to that of disease risk such as metabolic syndromes (25, 26), chronic obstructive pulmonary disease (27-29), or even cardiovascular disease (25, 30). Given these suggestions, the mechanisms of disease are necessities to prevention in lieu of treatment or to slow progression at the least. Commonly, EP initiate oxidative stress (30, 31) which, in turn, alters the normal cellular function of tissues as redox reactions occur within all cells (32).

Contrary to initial thoughts of what EP can be, natural chemicals such as formaldehyde (FA) as well as cigarette smoke, which can contain particulate matter (33), are considered air EP. Given the gaseous form of the aforementioned EP, potential for exposure to the pollutants is increased as they

dilute into fluids (34). With exposure of EP being gaseous, inhalation causes the respiratory system to be readily insulted (35) as well as diffuse EP into the blood (36) and increase oxidative stress (31, 37-41). Commonly, oxidative stress is induced by free radicals which later affects lipid and protein structures (31). Therefore, assessment of human physiology is essential to understanding how the correlations between EP and chronic disease are possible and how it is that humans may be affected so that appropriate precautions can be taken for a healthier life.

Chapter 2

Formaldehyde (FA) is a Group 1 carcinogen that has many applications in the preservation of biological materials given its fungicidal and bactericidal properties (42). While FA has many applications, the simplicity and size tend to allow for FA to disrupt protein and membrane organization. Therefore, the likelihood of FA disturbing human cells appears to be likely seeing as how the chemical can diffuse through mucosal membranes. Additionally, FA is found in either a liquid solution, known as formalin, or as a volatile organic compound in the air being breathed. The inspiration of FA has been known to cause irritation of the epithelial linings of the airways (39, 43) further suggesting that damage is incurred by FA inspiration.

The solution to preserving materials is, there in, the problem to basic physiological function. To gain perspective, hypothesis testing on experimental procedures would elucidate on the extent to which normal physiological function may be impaired, thus gaining traction for prevention of EP.

Vascular Dysfunction and Oxidative Stress Caused by

Acute Formaldehyde Exposure in Female Adults

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Keywords: Formaldehyde, Flow-Mediated Dilation, Passive Limb Movement, Vascular Function, Oxidative Stress

Word Count: 5,602

ABSTRACT

Introduction: Formaldehyde (FA) is a common, volatile organic compound used in organic preservation with known health effects of eye, nose, and throat irritation linked to oxidative stress and inflammation. Indeed, long-term FA exposure may provoke skin disorders, cancer, and cardiovascular disease. However, the effects of short-term FA exposure on the vasculature have yet to be investigated. **Purpose:** We sought to investigate the impact of an acute FA exposure on: 1) macrovascular function in the arm (brachial artery flow-mediated dilation, FMD), 2) microvascular function in the arm (brachial artery reactive hyperemia, RH) and leg (common femoral artery, supine passive limb movement, PLM), and 3) circulating markers of oxidative stress (xanthine oxidase, XO; protein carbonyl, PC; and malondialdehyde, MDA) and inflammation (C-reactive protein, CRP). **Methods:** Ten (n=10) healthy females (23±1y) were studied before and immediately after a 90-minute FA exposure ([FA]: 197±79ppb) in cadaver dissection laboratories. **Results:** Brachial artery FMD% decreased following FA exposure (Pre-FA Exp: 9.41±4.21%, Post-FA Exp: 6.74±2.57%, p=0.043), and FMD/Shear decreased following FA exposure (Pre-FA Exp: 0.13±0.07AU, Post-FA Exp: 0.07±0.03AU, p=0.016). The area under the curve for brachial artery RH (Pre-FA Exp: 481±191ml, Post-FA Exp: 499±165ml) and common femoral artery PLM (Pre-FA Exp: 139±95ml, Post-FA Exp: 129±64ml) were unchanged by FA exposure (p>0.05). Circulating MDA increased (Pre-FA Exp: 4.8±1.3μM, Post-FA Exp: 6.3±2.2μM, p=0.047) while XO, PC, and CRP were unchanged by FA exposure (p>0.05). **Conclusion:** These initial data suggest a short FA exposure can adversely alter vascular function and oxidative stress, influencing cardiovascular health.

Word Count: 245

NEW AND NOTEWORTHY

This study was the first to investigate the implications of acute formaldehyde (FA) exposure on adult female vascular function in the arms and legs. The main findings from this study were a decrease in conduit vessel function without any alteration to microvascular function following a 90-minute FA exposure. Additionally, the oxidative stress marker malondialdehyde increased after FA exposure. Taken together, these results suggest acute FA exposure have deleterious implications for the vasculature and redox balance.

INTRODUCTION

Formaldehyde (FA) is volatile organic compound (VOC) which readily reacts with organic material (1). As a Group 1 carcinogen, the Centers for Disease Control describes FA as capable of disrupting proteins, making FA an exceptional bactericide and fungicide (2). Such uses of FA include organic material preservation, most notably in the timber industry (3), healthcare professions (4), and even in cadaver preservation common in medical education (5, 6), making it one of the most common and hazardous air pollutants (7). Exposure to FA can occur directly to the skin, yet FA typically is unintentionally inhaled, causing irritation (8) and potential cellular damage to the epithelial lining of conducting airways (9). Further, FA can readily cross the pulmonary alveolar membrane (10, 11) and elicit deleterious effects in the circulation, leading to cardiovascular pathologies and disease risk production (12) similar to other airborne pollutants (13).

Most evidence suggesting FA exposure can lead to cardiovascular impairments stems from cell culture and animal models. Human umbilical vein cell (HUVEC) studies suggest endothelial cells are particularly vulnerable to FA exposure and result in cellular damage (14, 15). Low- and high-level FA exposures in rat aortas both *in vivo* and *in vitro* exhibited a paradoxical vasoconstriction and vasodilation response, respectively (16), suggesting FA may have a direct, concentration-dependent effect on vascular function. Observations of oxidative stress and likely macrophage-induced inflammation in the rat lung following FA exposure (17, 18) suggest a peripheral effect of cardiovascular impairments could be secondary to an airborne exposure to FA. Collectively, these data provide evidence for FA to elicit numerous direct and indirect effects on vascular function, yet little is known regarding the ability of FA to induce vascular alterations in humans.

Among human epidemiological data, growing evidence has linked FA exposure to an increase in systemic oxidative stress. Several occupational industries including nursing (4), pathology wards (5), cosmetic industry (19), plastic lamination (9), and wood industry (3) have provided evidence for heightened oxidative stress among FA exposed workers. Airborne pollutant-induced increases in

oxidative stress have the ability to decrease nitric oxide (NO) bioavailability, resulting in worsened endothelial-dependent vasoreactivity (20) which may increase the risk of cardiovascular events (21, 22). While this has not been tested with FA exposure, we speculate that FA-induced increase in oxidative stress may act similarly as other airborne pollutants to induce endothelial and vascular dysfunction. Indeed, investigations into the potential effects of FA exposure on the human vasculature are critically needed to examine a potential link between FA exposure and vascular health.

Therefore, this study sought to assess the impact of acute FA exposure on vascular function using clinically relevant and well-established vascular assessments: brachial artery flow-mediated dilation (FMD) (21-23) and reactive hyperemia (RH) (24, 25) in the arm and passive limb movement (PLM) (26, 27) in the leg. Using a natural experimental approach, we hypothesized that acute FA exposure, such as that typically experienced in anatomy cadaver dissection laboratories, would decrease FMD, RH and PLM, thus providing evidence for the ability of FA to impact vascular function. Furthermore, circulating markers of oxidative stress (xanthine oxidase, XO; protein carbonyl, PC; and melanodialdehyde, MDA) and an inflammatory marker predictive of cardiovascular disease (C-reactive protein, CRP) (28) were determined as potential mechanisms of vascular dysfunction, which we hypothesized would increase in response to acute FA exposure. While sex-specific consequences of air pollution are contentious, analyses of respiratory modifications report a stronger effect of various air pollutants among females than males, regardless of menstrual phase (29). Likewise, exposure to pollution of fine particulates may have a larger impact on female life expectancy (30). These sex-specific decrements in health following airborne pollutant exposure may be associated with systemic oxidative stress and inflammation along with subsequent vascular dysfunction (31) and arterial stiffness (32). Hence, this initial study sought to focus on the effects of acute FA exposure among female adults.

METHODS

Study Population.

Ten female adults, who were beginning cadaver dissection courses wherein they would be exposed to FA, were recruited for this investigation from Appalachian State University and Elon University. Subjects were tested within the first two weeks of the laboratory start date to ensure minimal previous exposure to FA. The subjects received a single FA exposure 2 to 5 days prior to study participation as a familiarization to the laboratory setting but were otherwise naïve to the longer, 90-minute FA exposure in which they were in close proximity to FA sources. Exclusion criteria included any known cardiovascular disease, amenorrhea, current or past smokers who were regularly exposed to high amounts of VOCs in cigarette smoke (33), hormone replacement therapy other than oral contraceptive use, chronic antioxidant/anti-inflammatory use, or individuals who were pregnant or trying to become pregnant. All procedures were approved by Appalachian State University and Elon University Institutional Review Boards, and measurements were performed in a thermoneutral environment. The subjects provided informed consent in accordance with the standards outlined by the Declaration of Helsinki.

Study Procedures.

Subjects were tested in the morning in a fasted state, having abstained from caffeine, alcohol, and exercise for 24 hours prior to a 90-minute FA exposure. Testing occurred in a quiet, thermoneutral environment (692-736 mmHg barometric pressure, 22-23^{o} C temperature, 33-50% relative humidity). All procedures were performed with subjects lying supine for 20 minutes prior to testing. Data collection included FMD, RH, PLM, and a venous blood draw. Subjects were then instrumented with a chest-mounted FA sensor prior to entering the cadaver dissection laboratory for 90-minutes where they were actively dissecting cadavers which were embalmed with a 37% FA solution. During the time in the cadaver dissection laboratory, subjects actively took part in cadaver dissection activities, standing over the FA preserved human donors which remained on stainless-steel cadaver tables at waist height.

Within the laboratories, subjects wore nitrile or latex gloves and garments to cover arms and legs to further prevent FA exposure directly to the skin, although masks were not worn. Following FA exposure, subjects left the cadaver laboratory and proceeded to the testing room where they were reassessed 29 ± 11 minutes after the 90-minute FA exposure. Study procedures were ordered similarly prior to and following FA exposure to maintain standardization.

Formaldehyde Sensors. Subjects wore FA sensors (FM-801 Formaldehyde meter, Graywolf Sensing Solutions, LTD, Annacotty, Ireland) anteriorly on a chest harness in an effort to measure individual FA exposure levels, due to personal exposure levels often being higher than ambient room FA concentrations, especially during cadaver dissections when FA is more readily emitted from cadavers to close proximity individuals (34, 35). The FA sensors use photoelectric photometry with accurate readings to 10 ppb. Airborne FA levels were measured using photoelectric photometry (407-424nm) as 30-minute averages for continuous data-monitoring. Sensors were calibrated for >60 minutes prior to FA exposure in the testing room according to manufacturer's instructions. FA levels were also recorded in the testing room where FMD, RH, PLM, and blood draw measurements were conducted before and after subject FA exposures in the cadaver dissection laboratory which were in the same building as the FA exposure to minimize distance traveled between repeated measurements.

Each anatomy laboratory was assessed for FA exposure levels within the past year using FA passive monitoring badges (Sensor Safety Products, Raleigh, NC). The Appalachian State University anatomy laboratory was built 1 year prior to this study taking place, is 95 square meters, and has downdraft cadaver dissection tables with in-table ventilation to draw air down under the cadavers and out of the room and building through a ventilation system with 100% fresh air turn over 12.5 times per hour. The Elon University School of Health Sciences anatomy laboratory was built 9 years prior to this study taking place, is 201 square meters, and has a ventilation system with 100% fresh air turn over 13 times per hour. Appalachian State University anatomy laboratory was tested within 4 months of this study taking place with FA levels below the acceptable OSHA permissible exposure limits of 750 ppb

for an 8-hour time-weighted average, and did not exceed the NIOSH Recommended Exposure Limit of 16 ppb for a 10-hour time-weighted average. Elon University School of Health Sciences anatomy laboratory was tested within 7 months of this study taking place with FA levels below the acceptable OSHA permissible exposure limits of 750 ppb for an 8-hour time-weighted average, but exceeded the NIOSH Recommended Exposure Limit of 16 ppb for a 10-hour time-weighted average. These results are consistent with low level exposure in an anatomy laboratory setting.

Experimental Measurements.

Brachial artery FMD and RH. Brachial artery FMD and RH measurements were obtained from the right brachial artery using current guidelines (36). Baseline measurements of the right brachial artery diameter and blood velocity were taken for 1 minute using a Doppler ultrasound system (GE Logiq eR7 and L4-12T-RS transducer, GE Medical Systems, Milwaukee, WI). Sample volume was optimized in relation to vessel diameter and centered within the vessel for each subject. Measurements of brachial artery diameter and velocity were obtained with the Doppler ultrasound in duplex mode with B-mode imaging frequency of 12 MHz and Doppler frequency of 4 MHz. An angle of insonation of ≤60° (37) was achieved for all measurements. Immediately after baseline measurements, a blood pressure cuff, placed distal to the elbow, was rapidly inflated to 250 mmHg for 5 minutes. The blood pressure cuff was rapidly deflated, and brachial artery diameter and velocity were recorded for 2 minutes. Brachial artery diameter, blood velocity, blood flow, shear rate, and RH were analyzed offline for continuous second-by-second measurements (Cardiovascular Suite version 4.0, Quipu, Pisa, Italy). Blood flow was determined as: $Blood\ Flow = [\pi\,(\text{arterial diameter} / 2)^2] \cdot \text{blood velocity}$, where blood velocity was obtained as the time average mean on the Doppler ultrasound.

Passive Leg Movement. Following brachial artery FMD procedures, resting, supine brachial artery blood pressures [systolic blood pressure, SBP; diastolic blood pressure, DBP; pulse pressure, PP, as the

difference between SBP and DBP; and mean arterial pressure, MAP, as $MAP = \left(\frac{2}{3} * DBP\right) + \left(\frac{1}{3} * SBP\right)$] and heart rate were obtained to determine resting central hemodynamics and to calculate vascular conductance as blood flow relative to MAP. Following these measurements of supine central hemodynamics, supine PLM was performed using current guidelines (26). While in the supine position with the subject's left leg supported on a stool and right leg supported by a research team member at heart level, baseline measurements of the common femoral artery diameter and blood velocity, at least 3 cm proximal the femoral artery bifurcation, were recorded for 1 minute prior to passive limb movement using similar Doppler ultrasound system settings used for the brachial artery FMD procedure. Immediately following baseline measurements, the research team member supporting the right thigh and ankle manually moved the knee joint one time through 90º range of motion, flexion-extension, at 1 Hz while common femoral artery diameter and blood velocity were recorded for one minute after the movement. Following this single passive limb movement (sPLM), measurements were repeated as the research team member manually moved the knee joint through 90º range of motion, flexion-extension, at 1 Hz continuously for one minute while common femoral artery diameter and blood velocity were recorded for one minute during this continuous passive limb movement (cPLM). Similar to the brachial artery FMD analyses, femoral artery diameter, blood velocity, and blood flow were analyzed offline for continuous second-by-second measurements (Cardiovascular Suite version 4.0, Quipu, Pisa, Italy). Resting, supine brachial mean arterial blood pressure (MAP) was used to normalize blood pressure as vascular conductance. The peak change in blood flow ($\Delta Peak_{BF}$) and vascular conductance ($\Delta Peak_{VC}$) along with the 60-second area under the curve (AUC 60s) were determined as indices of microvascular function (26).

Blood Draw. Blood draws were performed by a certified phlebotomist following vascular measurements. Plasma were separated by centrifugation and stored at -80 ºC until analysis. Biochemical assays were performed according to manufacturer's instructions in duplicate for the

oxidative stress biomarkers XO using a fluorometric assay (Cayman Chemical, Ann Arbor, MI, #10010895), PC using a colorimetric assay (Cayman Chemical, Ann Arbor, MI, #10005020), and MDA using a colorimetric assay (Cayman Chemical, Ann Arbor, MI, #10009055) as well as the inflammatory biomarker CRP using an enzyme-linked immunosorbent assay (Cayman Chemical, Ann Arbor, MI, #10011236).

Statistical Analysis. Statistics were performed using commercially available software (IBM SPSS Statistics version 26, Armonk, NY, USA). Paired two-tailed student's t-tests were performed, and data were checked for normality using Shapiro Wilk's Test and boxplots. Effect sizes are reported using Cohen's d_{av}. Statistical significance was specified at $p < 0.05$. Subject characteristics and variables of interest are expressed as mean ± SD. As an exploratory *Post Hoc* analysis, Pearson correlations were performed for the association between significant markers of vascular dysfunction and biomarkers of oxidative stress and inflammation.

RESULTS

Subject Characteristics. Subject characteristics of all ten subjects are presented in **Table 1**. One of the females who underwent FMD and RH testing did not undergo PLM or blood draw measurements.

FA Exposure. The average FA exposure among 9 subjects who wore the FA sensors during the 90-minute FA exposure was 197 ± 79 ppb, with 30-minute average FA levels ranging from 20 to 356 ppb. The lowest 30-minute FA exposure averaged 152 ± 78 ppm. The highest 30-minute FA exposure averaged 247 ± 90 ppb. Among the 30-minute average FA levels, 5 of the 9 measured subjects exceeded an FA exposure of 250 ppb during the 90-minute FA exposure time period. FA concentrations in the testing room were below 10 ppb which was the lowest detectable level among the five separate FA sensors utilized at any given time.

Brachial Artery FMD. Measurements of brachial artery FMD are presented in **Figure 1** and **Table 2**. Baseline diameter did not change due to FA exposure (p = 0.318, Cohen's d_{av} = 0.38). There was a tendency for the peak diameter to decrease following FA exposure (p = 0.054, Cohen's d_{av} = 0.70). However, the absolute change in diameter was not different following FA exposure (p = 0.371, Cohen's d_{av} = 0.49). The time to peak diameter did not change due to FA exposure (p = 0.515, Cohen's d_{av} = 0.35). The sum of shear experienced at peak diameter increased following FA exposure (p = 0.0.35, Cohen's d_{av} = 0.82) (**Table 2**). The peak change in diameter expressed as a percent, FMD% (Pre-FA Exp: 9.41 ± 4.21 %, Post-FA Exp: 6.74 ± 2.57 %, p = 0.043, Cohen's d_{av} = 0.79) and FMD normalized to the shear rate (Pre-FA Exp: 0.13 ± 0.07 AU, Post-FA Exp: 0.07 ± 0.03 AU, p = 0.016, Cohen's d_{av} = 1.20) decreased following FA exposure (**Figure 1A** and **Figure 1B**, respectively).

Brachial Artery Reactive Hyperemia. Blood flow measurements from before and after cuff occlusion are shown in **Figure 2**. Brachial artery blood flow prior to cuff occlusion was unchanged by FA exposure (Pre-FA Exp: 86.4 ± 18.5 ml·min^{-1}, Post-FA Exp: 97.5 ± 60 ml·min^{-1}, p = 0.536, Cohen's d_{av}

= 0.53). Likewise, the blood flow response to the 5-minute cuff occlusion, as assessed by AUC, was unchanged by FA exposure (Pre-FA Exp: 481 ± 191 ml, Post-FA Exp: 499 ± 165 ml, $p = 0.831$, Cohen's $d_{av} = 0.10$).

Supine Central Hemodynamics. Nine out of the ten subjects underwent supine testing for central hemodynamics in preparation for PLM testing. Blood pressure and heart rate measurements from before and after FA exposure are shown in **Table 3**. Resting supine SBP, DBP, PP, MAP, and HR were unchanged by FA exposure ($p > 0.05$).

Passive Leg Movement. Nine out of the ten subjects underwent PLM testing prior to and following FA exposure. Measurements of sPLM are presented in **Table 4** and **Figure 3**. Common femoral artery blood flow or vascular conductance at baseline did not change due to FA exposure ($p > 0.05$) (**Table 4**). Peak blood flow, peak vascular conductance, the change in blood flow from baseline to peak, and the change in vascular conductance from baseline to peak were not altered by FA exposure ($p > 0.05$) (**Table 4**). The blood flow response to the sPLM, as assessed by AUC 60s (Pre-FA Exp: 139 ± 95 ml, Post-FA Exp: 129 ± 64 ml, $p = 0.824$, Cohen's $d_{av} = 0.13$) and vascular conductance response to the sPLM, as assessed by AUC 60s (Pre-FA Exp: 1.91 ± 1.28 ml·mmHg^{-1}, Post-FA Exp: 1.41 ± 0.74 ml·mmHg^{-1}, $p = 0.376$, Cohen's $d_{av} = 0.50$) were unchanged by FA exposure (**Figure 3**).

Measurements of cPLM are presented in **Table 4** and **Figure 4**. Common femoral artery blood flow and vascular conductance at baseline did not change due to FA exposure ($p > 0.05$) (**Table 4**). Peak blood flow, peak vascular conductance, the change in blood flow from baseline to peak, and the change in vascular conductance from baseline to peak were not altered by FA exposure ($p > 0.05$) (**Table 4**). The blood flow response to the cPLM, as assessed by AUC 60s (Pre-FA Exp: 391 ± 220 ml, Post-FA Exp: 309 ± 195 ml, $p = 0.171$, Cohen's $d_{av} = 0.40$) and vascular conductance response to the cPLM, as assessed by AUC 60s (Pre-FA Exp: 4.54 ± 2.22 ml·mmHg^{-1}, Post-FA Exp: 3.37 ± 2.2 ml·mmHg^{-1}, $p = 0.162$, Cohen's $d_{av} = 0.53$) were unchanged by FA exposure (**Figure 3**).

Circulating levels of inflammation and oxidative stress. Nine out of the ten subjects had blood drawn prior to and following FA exposure. Measurements of circulating levels of oxidative stress and inflammation are presented in **Table 5**. Oxidative stress makers XO and PC did not change following FA exposure ($p > 0.05$). However, MDA increased following FA exposure ($p < 0.05$). The inflammatory marker CRP did not change following FA exposure ($p > 0.05$). The mean intra-assay coefficient of variation for all analyzed biomarkers revealed good reproducibility across duplicate samples (XO: 11.0%, PC: 4.3%, MDA: 7.1%, and CRP: 4.1%). The Pearson correlation between the change in FMD% from Pre-FA to Post-FA with the change in MDA from Pre-FA to Post-FA was not significant ($r = -0.101$, $P = 0.795$).

DISCUSSION

This study sought to identify the potential impact of an acute FA exposure on vascular function in the arms and legs of adult females, as well as the potential alterations to circulating levels of oxidative stress and inflammatory biomarkers. Our hypothesis was partially correct that FMD, as a percent change and when normalized to shear, decreased following a 90-minute FA exposure, suggesting vascular function in the brachial artery conduit vessel was impaired. However, as indicated by a lack of change to brachial artery RH, downstream vascular function in the small arterioles and microcirculation were not altered by the acute FA insult. Additionally, the lack of change to the common femoral artery blood flow response to either the sPLM or cPLM further corroborates the notion that the vasculature downstream of the conduit vessels were unaffected by the FA exposure. Despite a lack of change in microvascular function, we observed conflicting results in our circulating markers of oxidative stress without observable increases in either XO or PC but an increase in MDA. Furthermore, there was no change to the inflammatory marker CRP. This investigation represents some of the first data to identify a decrease vascular function in adult females following an acute FA exposure which may be attributable, at least in part, to an increase in oxidative stress.

Brachial Artery FMD with FA Exposure. The brachial artery FMD technique is a non-invasive, endothelial-dependent assessment of systemic vascular function (23) which strongly correlates with coronary vascular function (21) and is predictive of future cardiovascular events (22). Results from HUVEC studies suggest FA exposure can damage endothelial cells (15), which may directly initiate plaque formation, oxidative stress, and vascular disorders (14). Additional investigations in isolated aortic rings of rats have revealed a potential role of endothelin-induced vasoconstriction in response to low FA incubation (<300 μM), while higher levels of FA incubation (>500 μM) may decrease NO bioavailability (16). These studies highlight the potential impact of FA exposure to endothelial cells and the vasculature, while also stressing the importance of translational investigations in human vascular health. In the current investigation, the 3% decrease in brachial artery FMD is clinically

meaningful, as every 1% decrease in brachial artery FMD% is associated with a ~13% increased risk of cardiovascular events such as heart attack, stroke, or death (38). These observations, representing the first data to address the potential contribution of FA exposure to alter upper extremity vascular function in humans, suggest a rapid decrement in vascular function following a relatively short bout of FA exposure which have ramifications for cardiovascular health, even among young healthy individuals. Future research should certainly discern whether these decrements are shared among other populations, most notably individuals at higher risk for cardiovascular disease who may ultimately show a greater FA-induced decrement or among those with an already compromised vasculature, such as individuals with hypertension or heart failure. Furthermore, dose, duration, and repeated exposures of FA are needed to further understand the potential impact of FA to vascular function.

Brachial Artery Reactive Hyperemia with FA Exposure. Whereas brachial artery FMD provides an assessment of vascular function in the conduit artery, brachial artery RH provides an index of microvascular function which is inversely related to cardiovascular disease risk (24) and is predictive of future cardiovascular events in healthy and diseased populations (25). The lack of change in brachial artery RH in the current investigation (**Figure 2**) alludes to a lack of dysfunction among the small arterioles or microvasculature shortly after an acute FA exposure. Microvascular assessment of exposure to VOCs such as cigarette smoke are contentious. While some cross-sectional analyses of cigarette smokers compared to non-smokers have failed to show a change in brachial artery RH (39), acute exposure to VOCs in cigarette smoke have resulted in a decrease in brachial artery RH (40). One potential explanation for the discrepancy between our observed brachial artery vasodilatory impairments and lack of microvascular impairments with acute FA exposure may reside in the vasodilatory mechanisms contributing to each assessment. While brachial artery FMD is arguably a functional bioassay for endothelial-derived NO (41-43), the reactive hyperemic response to forearm cuff occlusion is only minimally influenced by NO (44, 45) and rather alternative pathways such as inwardly rectifying K^+ channels and Na^+/K^+ ATPase (46). If FA-induced oxidative stress is reducing

NO bioavailability and subsequent brachial artery FMD, perhaps the acute insult of oxidative stress observed in the current investigation is sparing microvascular dysfunction, albeit during this acute time period following FA exposure. Further, if oxidative stress has a delayed induction of inflammation which has been observed in animal models of FA exposure (17, 18), inflammation as a secondary insult may lag behind the increase in oxidative stress and lead to additional microvascular impairments hours to days following an acute FA exposure. Certainly, more work is warranted to discern if higher concentrations or durations of FA exposure may impose additional decrements downstream in the vascular tree and if prolonged effects following FA exposure may influence microvascular function.

Passive Limb Movement. The PLM maneuver has emerged as lower limb assessment of microvascular function (26) which may be attributable to a number of central and peripheral factors (27). While the sPLM elicits a minimal central hemodynamic response, the cPLM is thought to incorporate mechanoreflex type III/IV afferent feedback (47), thus providing sufficient time to increase cardiac output as an integrative response to the cPLM maneuver (48). This study represents the first investigation to address the potential contribution of FA exposure to alter lower limb vascular function, as assessed by the PLM maneuver. In the present investigation, a lack of change was observed in either the sPLM or cPLM maneuver when comparing the effect of FA exposure on either blood flow or vascular conductance (**Table 4**). Together, the sPLM and cPLM maneuvers provide insight into lower limb vascular function which corroborate our findings from the brachial artery RH in the arm, as both techniques assess microvascular function. Combined, these data suggest the acute effect of FA exposure on the vasculature may have regional rather than global distinctions among the vascular tree.

Oxidative Stress and Inflammation with FA Exposure. Oxidative stress is a primary mechanism by which airborne pollutants are thought to inflict global health decrements (49). The axis of oxidative stress, inflammation, and vascular function has been well recognized, as a feedback loop of oxidative stress and inflammation which produce free radicals, diminish antioxidant capacity, uncouple eNOS

from superoxide anions, reduce NO bioavailability, and ultimately impair vascular function (50). However, many uncertainties exist regarding the interplay of redox balance and vascular function, especially among FA exposure.

Oxidative stress plays a central role in the response to acute airborne pollutants. Studies from smokers (51) and bus drivers (52) exposed to VOCs provide evidence for increased circulating levels of the primary superoxide generator XO as well as protein oxidation biomarker PC, respectively. However, investigations on the effect of human exposure to FA on XO and PC are lacking. As observed in the current investigation (**Table 5**), XO and PC were unaffected by an acute exposure to FA. These results suggest elevations in XO and PC are not mutually exclusive to all VOCs and may not follow similar patterns, albeit acutely, after exposure to FA. Further investigations may wish to investigate the effects of repeated bouts of FA exposure on these oxidative stress markers.

Epidemiological investigations also suggest increases in oxidative stress following FA exposure, especially among lipid oxidation. Nurses (4) and wood industry workers (3) exposed to FA experienced an increase in the lipid oxidation biomarker 15-F2t-isoprostane. Plastic laminate plant workers who are regularly exposed to FA had elevated levels of M1dG adducts, a biomarker of oxidative stress and lipid peroxidation (9). Similar to smokers regularly exposed to VOCs (51), exposure to FA yielded an increase in the global lipid peroxidation biomarker MDA among nurses (4), pathology ward workers (5), and cosmetic industry workers (19). The current investigation observed an increase in MDA (**Table 5**) with acute exposure to FA, which suggests the potential for FA to rapidly increase lipid oxidation. Yet, this change in MDA was not associated with a change in vascular function. Future studies should certainly discern if a time course effect exists among these biomarkers and whether oxidative stress persists hours to days following initial FA exposure.

While important to consider the effects of oxidative stress on vascular function, the balance of antioxidant defenses combating reactive oxygen species should be considered when further evaluating the potential impact of FA exposure on the vasculature. Concentrations of the antioxidant glutathione, which can oxidize FA to formic acid (53), can decrease in response to a 4-week FA exposure in rats

(54), suggesting antioxidants may be expendable to prevent a rise in reactive oxygen species. Several investigations using animal models provide evidence for antioxidant administration, such as rose oil (55), lavender oil (56), or melatonin (56) to improve redox balance and have protective effects against FA-induced cellular damage. Further investigations should certainly discern between the ability of endogenous or exogenously administered antioxidants to modulate oxidative stress and whether antioxidant levels diminish due to acute, chronic, and repeated FA exposures which may have further implications for cardiovascular health.

In addition to oxidative stress, FA can induce an inflammatory response. Studies in rats have identified an increase in leukotriene B4, thromboxane B2, interleukin-1, interleukin-6 and vascular endothelial growth factor which may play a role in the inflammatory response to FA exposure (17, 18). Additionally, administration of the antioxidants vitamins C, E, and apocynin in rats was able to prevent this rise in FA-induced inflammation (18), suggesting a role of oxidative stress in generating lung inflammation which may also be present systemically. Furthermore, CRP is a sensitive marker of inflammation, tissue damage, and infection (57) which is an independent risk factor for cardiovascular disease events (28, 58). Long-term exposure to VOCs in cigarette smoke increases CRP among smokers which may be reversable with years of smoking cessation (59). This suggests CRP may be influenced by other VOCs such as FA. Despite this evidence for enhanced inflammatory responses to FA and other VOCs, the current investigation failed to detect a change in CRP following an acute FA exposure for 90 minutes (**Table 5**). Over a long enough time period, oxidative stress may exacerbate inflammation and reduce vascular function (50). Yet, more work is needed to investigate the effects of higher FA concentrations, repeated exposures, and long-lasting consequences which may enhance inflammatory pathways for hours to days following an FA exposure and may contribute to additional decrements in vascular function.

Limitations. We recognize the nature of this natural experimental design may have had several confounding variables. Mental stress may enhance cortisol levels which are negatively associated with

vasodilation during the traditional FMD technique, and these heightened cortisol levels can be ameliorated with administration of the antioxidant Vitamin C (60), suggesting an association between oxidative stress, autonomic outflow, and vascular function. Likewise, the enhanced sympathetic nervous system response to the cold pressor test (61) may influence the traditional FMD (62) as well as sustained, exercise-induced increase in shear rate (63). Further, mental stress caused by decreased sleep quality has been well documented as influencing vascular function through alterations in vascular resistance and conduit artery shear stress profiles (64-66). While stress cannot be ruled out as a confounding variable in the current investigation, the lack of change in supine central hemodynamics in response to FA exposure (**Table 3**) may suggest a lack alteration to autonomic outflow which may otherwise influence vascular function in the current study. Future investigations may wish to control for such forms of mental stress, especially when assessing repeated days of FA exposure commonly experienced in occupational settings. Further, the females studied in the current investigation had variations in contraceptive use and menstrual cycle fluctuations which may contribute to variable responses to airborne pollutants, as the cardio-protective attributes of estrogen should not be overlooked (67, 68). However, previous accounts of endothelial function were not altered across the menstrual cycle and with oral contraceptive use (69) which suggests the current findings may not be confounded by the menstrual cycle. While it is enticing to monitor plasma FA levels, FA has a half-time of only 1-1.5 minutes in plasma (70), making monitoring FA metabolites, such as formate salts in the urine, the next best option (19), despite levels of detection of formate being questionable during relatively low levels of FA exposure (71). Additionally, as standing may be beneficial over prolonged sitting for micro- and macrovascular function (72-74), the effect of FA exposure on the vasculature among individuals who were standing over cadavers for 90-minutes may have been more pronounced had the subjects been sitting. Certainly, more work is needed to identify if postural or light physical activity may influence these vascular alterations. Given the acute nature of the current investigation, additional time points after FA exposure are needed to identify any time course effect in future investigations.

Perspectives. Many occupations encounter FA on a daily basis including those in the lumber and cosmetic industries, as well as those in healthcare professions, the latter of which often encounter high FA levels in their cadaver-based anatomy dissection training. While modern cadaver laboratories utilize exhaust systems within, above, or near cadaver tables, the FA exposure levels may remain 2-3 times higher than average indoor FA levels and may exceed OSHA and NIOSH guidelines (6, 34). In the current investigation, the use of personal FA monitors was essential to identify a ~20-fold variation in 30-minute average FA exposure levels among individuals who were in the same working environment while performing the similar tasks. In practice, the level of FA exposure to those who are instructing, learning, and working is of great theoretical importance and should not be assumed to be equal within similar working environments. Indeed, more work is needed to investigate repeated and duration-dependent effects of FA exposure on the vasculature which many individuals may be exposed to for several hours over the course of years, possibly having additive effects on cardiovascular disease risk. Furthermore, future work may wish to investigate the potential acute or chronic interventions to ameliorate these effects of FA exposure on vascular function, especially among those who are regularly exposed to this common occupational hazard.

Conclusion. To the best of our knowledge, this is the first study to identify the potential effects of an acute FA exposure on peripheral vascular function in female adults, which may have ramifications for cardiovascular health. A relatively short, 90-minute FA exposure, which was within OSHA recommended FA exposure concentration limits (75), was sufficient to diminish brachial artery FMD and increase oxidative stress immediately after FA exposure among healthy female adults. While further research is surely necessary to identify additional impacts of various FA exposure concentrations and durations among both sexes, aging individuals, and those who are more susceptible to cardiovascular disease, these data provide guidance for the cautionary use of FA within indoor environments.

FUNDING

Partially funded by Faculty Research and Development Award through Elon University School of Health Sciences (C.B.).

ACKNOWLEDGEMENTS

We would like to thank Ashley Goodman, Ph.D. at Appalachian State University for initializing this research collaboration between Appalachian State University and Elon University.

AUTHOR CONTRIBUTIONS

J.S., J.C., C.B., S.R., conceived and designed research; M.A., N.S., L.K., S.R. performed experiments; M.A., J.S., N.S., L.K., S.R. analyzed data; M.A., J.S., N.S., L.K., J.C., C.B., S.R. interpreted results of experiments; N.S., S.R. prepared figures; M.A., J.S., N.S., S.R. drafted manuscript; M.A., J.S., N.S., L.K., J.C., C.B., S.R. edited and revised manuscript; M.A., J.S., N.S., L.K., J.C., C.B., S.R. approved final version of manuscript.

CONFLICT OF INTEREST

No conflicts of interest, financial or otherwise, are declared by the authors.

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FIGURE LEGEND

Figure 1. Brachial artery flow-mediated dilation. Brachial artery flow-mediated dilation (FMD) expressed as percent change (A; $p = 0.043$, Cohen's $d_{av} = 0.79$) and normalized to shear (B; $p = 0.016$, Cohen's $d_{av} = 1.20$) before formaldehyde exposure (Pre-FA Exp) and after (Post-FA Exp). Individual trends shown in grey bars, n=10. Paired two-tailed student's t-tests were performed between Pre-FA and Post-FA Exp. * $p<0.05$. Data are Mean ± SD.

Figure 2. Brachial artery reactive hyperemic response to cuff occlusion. Brachial artery reactive hyperemic response to cuff occlusion expressed as absolute blood flow response before formaldehyde exposure (Pre-FA Exp) and after (Post-FA Exp). Inset depicts area under the curve for the 120 seconds after cuff occlusion (AUC 120s) with individual trends shown in grey bars, n=10, $p = 0.831$, Cohen's $d_{av} = 0.10$. Paired two-tailed student's t-tests were performed between Pre-FA and Post-FA Exp. Data are Mean ± SD.

Figure 3. Single passive limb movement. Common femoral artery blood flow response to a single passive limb movement (A; $p = 0.824$, Cohen's $d_{av} = 0.13$) before formaldehyde exposure (Pre-FA Exp) and after (Post-FA Exp). Vascular conductance response to a single passive limb movement (B; $p = 0.376$, Cohen's $d_{av} = 0.50$). Insets depict area under the curve for the 60 seconds after sPLM (AUC 60s) with individual trends shown in grey bars, n=9. Paired two-tailed student's t-tests were performed between Pre-FA and Post-FA Exp AUC. Data are Mean ± SD.

Figure 4. Continuous passive limb movement. Common femoral artery blood flow response to continuous passive limb movements (A; $p = 0.171$, Cohen's $d_{av} = 0.40$) before formaldehyde exposure (Pre-FA Exp) and after (Post-FA Exp). Vascular conductance response to continuous passive limb movements (B; $p = 0.162$, Cohen's $d_{av} = 0.53$). Insets depict area under the curve for the 60 seconds

during cPLM (AUC 60s) with individual trends shown in grey bars, n=9. Paired two-tailed student's t-tests were performed between Pre-FA and Post-FA Exp AUC. Data are Mean ± SD.

Table 1. Subject characteristics.

Subject Characteristics		**95% CI**
Subjects, n	10	
Age, y	23 ± 1	22, 24
Race, n		
Caucasian	8	
African American	0	
Hispanic	2	
BMI, kg/m^2	22.9 ± 2.5	21, 24
Physical Activity		
Frequency, d	3.4 ± 1.1	2.7, 4.1
Duration, min/d	44 ± 14	35, 53
Contraceptive Use, n	8	

CI, confidence interval. Data are Mean ± SD.

Table 2. Flow-mediated dilation.

	Pre-FA Exp	95% CI	Post-FA Exp	95% CI	ΔPre-FA Exp to Post-FA Exp	95% CI	Effect Size	*p*-value
Baseline diameter, mm	3.36 ± 0.44	3.09, 3.63	3.21 ± 0.34	3.00, 3.42	-0.14 ± 0.43	-0.41, 0.13	0.38	0.318
Peak diameter, mm	3.72 ± 0.46	3.44, 4.00	3.47 ± 0.25	3.32, 3.63	-0.25 ± 0.36	-0.47, -0.03	0.70	0.058
Change in diameter, mm	0.36 ± 0.28	0.19, 0.53	0.26 ± 0.13	0.18, 0.34	-0.10 ± 0.34	-0.31, 0.11	0.49	0.371
Time to peak diameter, sec	56 ± 21	43, 69	64 ± 25	49, 80	8 ± 37	-15, 31	0.35	0.515
Sum of shear at peak, AU	8.2 ± 3.1	6.3, 10.1	10.7 ± 3.0*	8.8, 12.6	2.5 ± 3.2	0.5, 4.5	0.82	0.035

n = 10. Paired two-tailed student's t-tests were performed along with Cohen's d_{av} effect size. AU, arbitrary units; CI, confidence interval. * $p<0.05$.

Data are Mean ± SD.

Table 3. Supine central hemodynamics.

	Pre-FA Exp	95% CI	Post-FA Exp	95% CI	ΔPre-FA Exp to Post-FA Exp	95% CI	Effect Size	*p*-value
Systolic blood pressure, mmHg	106 ± 8	101, 111	108 ± 4	105, 111	2 ± 8	-3, 7	0.33	0.591
Diastolic blood pressure, mmHg	76 ± 4	73, 79	77 ± 4	74, 80	1 ± 4	-2, 4	0.25	0.546
Pulse pressure, mmHg	30 ± 5	27, 33	31 ± 4	28, 34	1 ± 6	-3, 5	0.22	0.761
Mean arterial pressure, mmHg	89 ± 5	86, 92	91 ± 4	88, 94	2 ± 6	-2, 6	0.46	0.439
Heart rate, bpm	70 ± 12	62, 78	74 ± 11	67, 81	3 ± 12	-5, 11	0.35	0.411

n = 9. Paired two-tailed student's t-tests were performed along with Cohen's d_{av} effect size. BF, blood flow; CI, confidence interval. Data are Mean ± SD.

Table 4. Passive limb movement.

	Pre-FA Exp	95% CI	Post-FA Exp	95% CI	ΔPre-FA Exp to Post-FA Exp	95% CI	Effect Size	*p*-value
Single PLM								
Baseline BF, ml/min	522 ± 208	386, 658	470 ± 206	335, 605	-53 ± 145	-148, 42	0.25	0.304
Peak BF, ml/min	968 ± 251	804, 1130	874 ± 316	668, 1080	-93 ± 181	-211, 25	0.33	0.160
$\Delta Peak_{BF}$, ml/min	445 ± 117	369, 521	404 ± 169	294, 514	-40 ± 202	-172, 92	0.29	0.565
Baseline VC, ml/min/mmHg	5.8 ± 2.3	4.3, 7.3	5.0 ± 2.0	3.7, 6.3	-0.8 ± 1.5	-1.8, 0.2	0.37	0.159
Peak VC, ml/min/mmHg	10.7 ± 2.7	8.9, 12.5	9.4 ± 3.4	7.2, 11.6	-1.3 ± 1.8	-2.5, -0.1	0.43	0.061
$\Delta Peak_{VC}$, ml/min/mmHg	4.9 ± 1.3	4.1, 5.8	4.4 ± 2.0	3.1, 5.7	-0.5 ± 2.3	-2.0, 1.0	0.31	0.492
Continuous PLM								
Baseline BF, ml/min	513 ± 176	398, 628	547 ± 214	407, 687	35 ± 134	-53, 123	0.17	0.462
Peak BF, ml/min	1246 ± 324	1030, 1460	1200 ± 312	996, 1400	-45 ± 188	-168, 78	0.14	0.489
$\Delta Peak_{BF}$, ml/min	733 ± 252	568, 898	653 ± 214	513, 793	-80 ± 160	-185, 25	0.34	0.173
Baseline VC, ml/min/mmHg	5.7 ± 1.8	4.5, 6.9	5.9 ± 2.2	4.5, 7.3	0.2 ± 1.5	-0.8, 1.2	0.10	0.683
Peak VC, ml/min/mmHg	13.8 ± 3.4	11.6, 16.0	13.0 ± 3.5	10.7, 15.3	-0.8 ± 2.1	-2.2, 0.6	0.23	0.298
$\Delta Peak_{VC}$, ml/min/mmHg	8.1 ± 2.7	3.6, 9.9	7.1 ± 2.5	5.5, 8.7	-1.0 ± 1.8	-2.2, 0.2	0.38	0.136

n = 9. Paired two-tailed student's t-tests were performed along with Cohen's d_{av} effect size. BF, blood flow; CI, confidence interval; VC, vascular conductance. Data are Mean ± SD.

Table 5. Circulating levels of oxidative stress and inflammation.

	Pre-FA Exp	95% CI	Post-FA Exp	95% CI	ΔPre-FA Exp to Post-FA Exp	95% CI	Effect Size	*p*-value
Oxidative Stress								
Xanthine oxidase, µU/ml	15.2 ± 24.6	-0.9, 31.3	20.6 ± 36.8	-3.4, 44.6	5.4 ± 25.3	-11.1, 21.9	0.18	0.540
Protein carbonyl, pmol/ml	7.3 ± 5.2	3.9, 10.7	6.0 ± 6.4	1.8, 10.2	-1.3 ± 5.5	-4.9, 2.9	0.22	0.493
Malondialdehyde, µM	4.8 ± 1.3	4.0, 5.7	6.3 ± 2.2 *	4.9, 7.7	1.5 ± 1.9	0.3, 2.7	0.86	0.047
Inflammation								
C-reactive protein, pg/mol	1684 ± 1232	879, 2490	1989 ± 1335	1120, 2860	304 ± 803	-221, 829	0.24	0.288

n=9. Paired two-tailed student's t-tests were performed along with Cohen's d_{av} effect size. CI, confidence interval. * $p<0.05$. Data are Mean ± SD.

Figure 1. Brachial artery flow-mediated dilation.

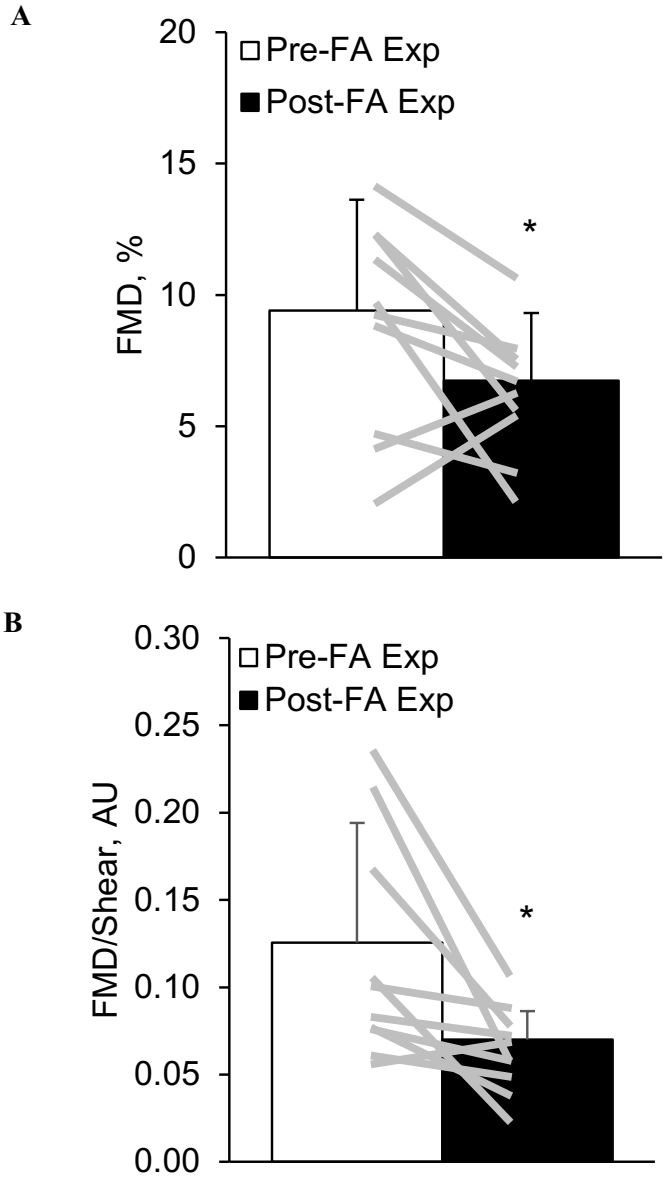


Figure 2. Brachial artery reactive hyperemic response to cuff occlusion.

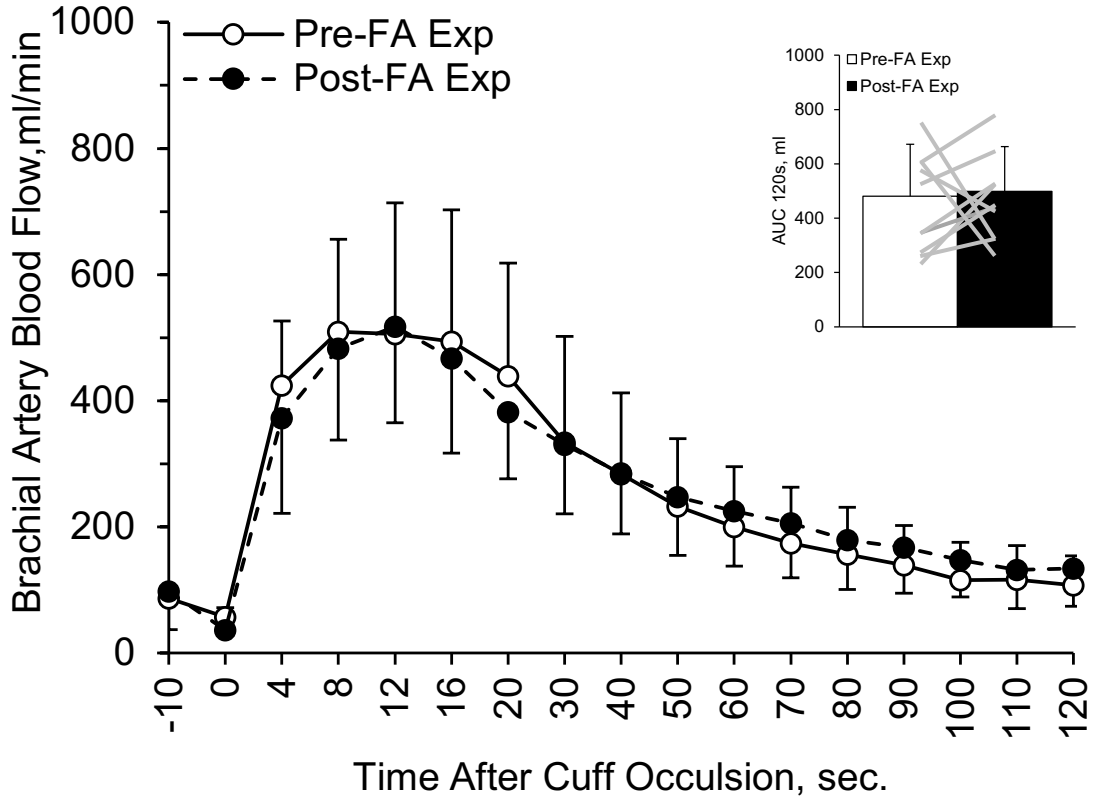


Figure 3. Single passive limb movement.

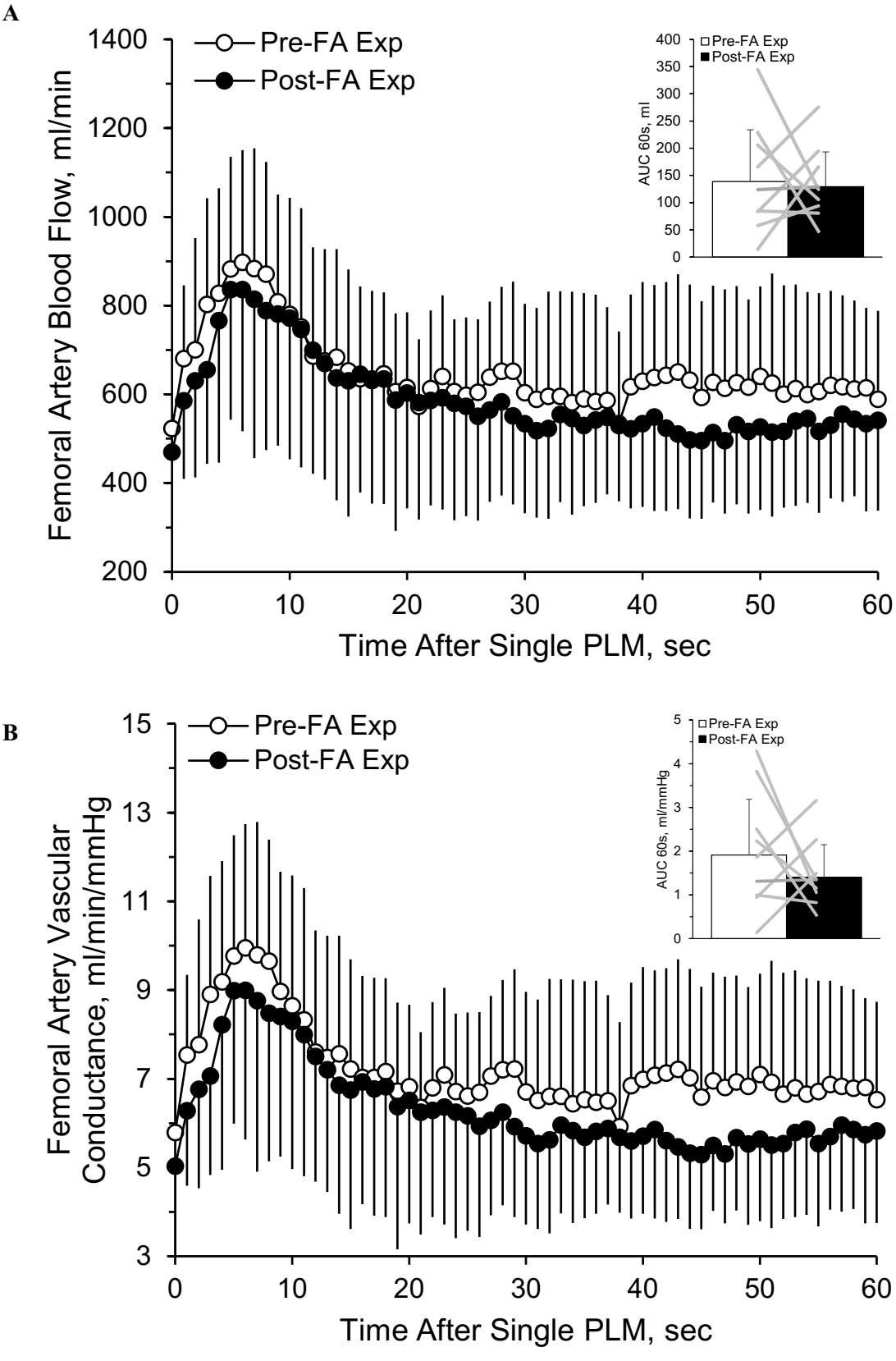
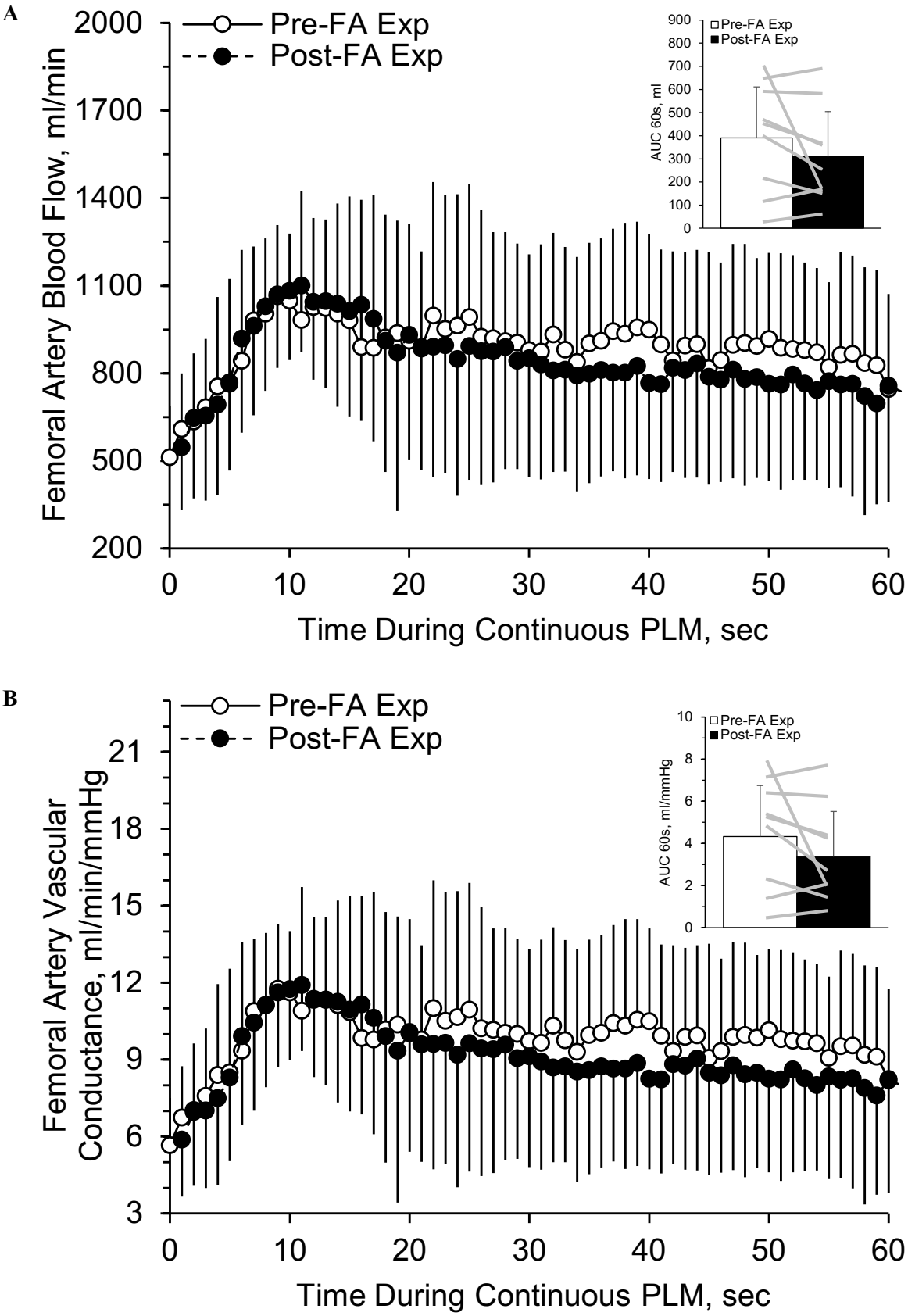


Figure 4. Continuous passive limb movement.



Title: **Pulmonary function following acute formaldehyde exposure in young adults**

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Running Title: Pulmonary Function following Acute Formaldehyde Exposure

Keywords: Spirometry, Biomarker

Word Count: 4315

ABSTRACT

Background: Formaldehyde (FA) is commonly utilized preservation agent contained within many household and industrial products, as well as in the solutions common to medical laboratories and mortuaries. Yet, FA is also a known carcinogen and pulmonary irritant.

Purpose: To investigate the effects of FA exposure during a cadaver dissection laboratory on pulmonary function and biomarkers of inflammation.

Methods: Students from two regional universities were recruited to perform pulmonary function testing and provided blood samples before and after following a 90-min cadaver dissection laboratory session. Spirometry was measured by having subjects complete forced vital capacity (FVC) and maximal voluntary ventilation (MVV) maneuvers following American Thoracic Society guidelines. The NHANES III dataset was used to calculate percent predicted values. Pre- and post-FA exposure data were subsequently compared.

Results: Before entering the laboratory, subjects (N=17; two males; 24±3yr; 24±4kg·m^{-2}) displayed normal pulmonary function, as indicated by the percent predicted values for FVC (97±10%), forced expiratory volume in one second (FEV_1; 96±11%), and MVV (102±16%). Subjects were exposed to 192 ± 124 ppb of FA over the course of the dissection period. Following the session, no changes were observed for any of the spirometric parameters examined (FVC, 0±3%Δ; FEV_1, 0±4%Δ; MVV, 0±0%Δ). Further, there were no correlations between the percent changes in FVC or FEV_1 and FA exposure concentrations as well as inflammatory biomarkers (r^2<0.06).

Conclusion: Acute exposure to FA over the course of a single dissection laboratory does not impair pulmonary function.

Abstract word count: 239

INTRODUCTION

Formaldehyde (FA) is a chemical commonly used during the production of many industrial and household products such as resins, paint, textiles, glues, cosmetics, paper products, particle board, etc. Additionally, FA-containing solutions are used within medical laboratories and mortuaries to preserve cadavers [1] due to its strong bactericidal, fungicidal, and insecticidal properties [2]. While the Occupational Health and Safety Administration (OSHA) has set permissible exposure limits for short- (15 min; 2 ppm) and long-term (8 hr; 0.75 ppm) exposures [3], many workers, as well as students enrolled in human cadaver dissection laboratories as part of their clinical education, may be exposed to levels of FA under the recommended limits but that still have been demonstrated to be sensitive to the eyes, nasal cavity, pharynx, and/or respiratory tract.

It is clear that chronic exposure to FA results in respiratory dysfunction [4-6] and is associated with greater risks of certain types of cancer[7] and overall mortality[8]. Additionally, repeated FA exposure in the workplace has been linked to decrements in pulmonary function in workers [4,9]; notable changes to forced vital capacity (FVC) and the forced expiratory volume in one second (FEV_1) have been documented following five years of chronic, high exposure[10]. Yet, the degree, if any, to which cardiorespiratory health is affected following acute FA exposure is less clear, especially when exposed to acceptable concentrations as determined by OSHA. Further, students involved in cadaver dissections within a gross anatomy course or actively engaged in medical laboratory activities may be exposed to levels of FA that impair normal respiratory function [11-13]. In contrast, a meta-analysis concluded that the potential changes to common spirometry measures were not physiologically meaningful [14]. The equivocal findings among investigations may be attributed to differences in the FA inhalation dosage (i.e., exposure concentrations, durations, and pulmonary ventilations) in subjects between each study, as well as between subjects within the same study. For example, depending on the level of involvement in the dissection activities, it is possible for two individuals within the same medical laboratory to be exposed to vastly different FA concentrations, and individual exposures likely will be greater than the mean room concentration [15]. Thus, if the individual

exposures are not accounted for or are completely unknown, interpretation of findings regarding respiratory function following FA exposure can be difficult at best or inaccurate at worst.

Studies have shown that FA exposures are commonly associated symptoms of respiratory distress (e.g. wheezing, productive cough, runny nose, headache) [13,14,16-18]. The immediate irritation to tissue mucosa is likely attributed to the high solubility of FA in water where it can deposit and be metabolized within neighboring cells. Yet, the exact mechanisms by which FA exposure can illicit changes to pulmonary function have not been fully delineated. It was recently demonstrated that FA modulates Ca^{2+} pathways in airway smooth muscle cells, which can result in airway narrowing [19]. Additionally, exposure to formaldehyde may lead to eosinophilia and greater cytokine release, leading to an inflammatory response that could contribute to bronchoconstriction [20]. Further, it has been hypothesized that disruption of the pathways involved in the relaxation of airway smooth muscle cells resulting in bronchodilation also may be altered [21]. Indeed, FA exposure has been linked to the development of symptoms characteristic of asthma in animal models [22,23], as well as to an increased risk of developing asthma in children and adults [24,25]. Furthermore, individuals with asthma may to be particularly susceptible to the respiratory consequences of acute FA exposure due to the already-heightened immune response [26]. Since we recently demonstrated that vascular dysfunction results from a single FA exposure bout, even when the FA exposure is within OSHA limits, and that the observed dysfunction corresponded with increased indicators of oxidative stress [27], we believe pulmonary function has the potential to be altered in conjunction with increased cytokine appearance in the blood.

The purpose of this study was to measure pulmonary function and circulating markers of inflammation in the before and after acute exposure to FA. A secondary purpose was to examine the relationships between the changes in pulmonary function parameters, circulating markers of inflammation, and individual FA concentration levels. We hypothesized that pulmonary function would be impaired, as evidenced by reductions in FVC and FEV_1, following a single acute exposure to FA within a medical laboratory. Additionally, we believed biomarkers of inflammation would be elevated as a result of the FA

exposure. Furthermore, we hypothesized that the degree of pulmonary function impairment, change in inflammatory mediators, and individual FA exposure concentrations would be related.

METHODS

Subjects

Individuals enrolled in a cadaver dissection laboratory course at one of two regional universities were recruited for participation. Subjects were studied during the first two weeks of the cadaver dissection course to minimize previous exposure to FA; the subjects received a single FA exposure 2 to 5 days prior to study participation as a familiarization to the laboratory setting but were otherwise naïve to the longer, 90-minute FA exposure. All subjects were at least 18 years of age. Exclusion criteria included any known cardiovascular or metabolic disease, current or past smokers, or individuals who were pregnant or trying to become pregnant. All procedures were approved by Appalachian State University and Elon University Institutional Review Boards. The subjects provided informed consent in accordance with the standards outlined by the Declaration of Helsinki.

Study Design

Following informed consent, all subjects completed a health history questionnaire. Subsequently, subjects completed pulmonary function testing (n = 17) and provided a blood sample (n = 12) before a 90-min cadaver dissection laboratory period (Pre). A subset of the subjects (n = 11) were randomly selected to wear FA sensors mounted to chest harnesses. During the time in the cadaver dissection laboratory, subjects actively took part in cadaver dissection activities, standing over the FA-preserved human donors, which remained on stainless-steel cadaver tables at waist height. During the dissection activities, subjects wore nitrile or latex gloves and garments to prevent FA contact with the skin. Subjects did not wear masks during the dissection period. Following the FA exposure period, subjects left the medical laboratory and proceeded to the physiological testing room where pulmonary function was reassessed and another blood sample was collected (Post).

Formaldehyde Concentrations

Subjects wore FA sensors (FM-801 Formaldehyde meter, Graywolf Sensing Solutions, LTD, Shelton, CT) anteriorly on a chest harness in an effort to measure individual FA exposure levels, since individual exposure levels are often higher than ambient room FA concentrations, especially during cadaver

dissection activities [15]. The sensors determined airborne FA levels using photoelectric photometry (407-424nm). Sensors were calibrated for >60 minutes prior to the FA exposure period. Subjects entered in the cadaver dissection laboratory where they remained for 90 min. Ambient air was continuously monitored for FA, and 30 min averages were reported. FA concentrations also were recorded in the physiological testing environment before and after the FA exposure period. The physiological testing environments and medical laboratories were located within the same buildings on each campus, which minimized the time and activity between the exposure period and reassessment.

Pulmonary Function Testing

Pulmonary function testing was performed according to American Thoracic Society recommendations using a portable, handheld spirometer (EasyOne Plus, ndd Medical Technologies, Andover, MA). Subjects were asked to perform a minimum of three FVC maneuvers. With a nose clip secured, subjects inspired to total lung capacity and were encouraged to forcefully expire to residual volume. Two maneuvers were required to meet the criteria of repeatability as defined by two FVC measurements within 0.150 L. The largest values for each of FVC and FEV_1 were selected for future comparisons. Additional parameters were selected from the single best maneuver (equal to the sum of FVC and FEV_1) and included peak expiratory flow (PEF), forced expiratory flows (FEF) over the midexpiratory range (25, 50, and 75% FVC), and the forced expiratory time (FET 100%). Following completion of the FVC maneuvers, subjects were asked to perform a maximum voluntary ventilation (MVV) test for 12 s, and measured volumes were multiplied by five to extrapolate the 12-s volume to a minute value. Participants were coached and encouraged throughout the MVV test. The largest value of two repeatable trials (<10% difference) was used for statistical comparison. Environmental conditions within each physiological testing environment were consistent over the course of the study period (Site 1: 730.1 ± 6.4 mmHg, 22.8 ± 0.5° C, 40 ± 8 %; Site 2: 692.0 ± 0.0 mmHg, 21.5 ± 0.5° C, 34 ± 1 %). All data are reported as absolute values (corrected to body temperature, pressure, saturated conditions) and as percentages of predicted (%pred) using the National Health and Nutrition Examination Survey (NHANESIII) data set[28].

Inflammatory Biomarkers

Blood samples were collected by a certified phlebotomist following pulmonary function testing. Plasma were separated by centrifugation and stored at -80°C until analysis. Interleukin-6 (IL-6; Cayman Chemical, Ann Arbor, MI; no.501030) and 15(S)-hydroxyeicosatetraenoic acid (15(S)-HETE; Cayman Chemical, Ann Arbor, MI; no.534721) were analyzed in triplicate using the enzyme-linked immunosorbent assay technique according to manufacturer's instructions. The coefficient of variation was < 10% (2.6 ± 2.1 %) for all samples. The mean concentrations of the triplicate samples were used in subsequent statistical comparisons.

Statistical Analysis

Statistical comparisons of pulmonary function and inflammatory biomarker measurements before and after FA exposure were completed using paired samples *t* tests (IBM, Armonk, NY). Pearson's correlations were calculated to assess the relationships between pulmonary function, blood biomarker, and FA concentrations. For all tests, statistical significance was set at $p < 0.05$. Descriptive data are reported as mean ± SD.

RESULTS

Subjects

Subject characteristics are reported in **Table 1.** Two male subjects participated, and their data are displayed separately for informative purposes only. Three of the subjects reported being diagnosed with asthma as children and were included in the study without assessment of airway reversibility.

Formaldehyde Concentrations

FA concentration over the full 90-min exposure period was 192 ± 124 ppb (minimum = 16 ppb; maximum = 352 ppb) for all subjects. However, 30-min averages ranged from as low as 10 ppb to as high as 473 ppb. Seven subjects were exposed to concentrations of at least 250 ppb during a single 30-min period. Individual readings are reported in **Table 2**.

Pulmonary Function Testing

FVC was maintained from before to after the 90-min FA exposure period (Pre: 3.93 ± 0.79 L, Post: 3.92 ± 0.86 L; $p > 0.05$). Measurements of FVC were 97 ± 10 %pred and 97 ± 9 %pred at the Pre and Post, respectively, physiological assessment time points. FEV_1 was 96 ± 11 %pred before and 96 ± 11 %pred after the FA exposure period. Thus, FEV_1 was unchanged as a result of the exposure to FA (Pre: 3.33 ± 0.66 L, Post: 3.33 ± 0.68 L; $p > 0.05$). Additional pulmonary function measurements are reported in **Table 3.** Like the observations of FVC and FEV_1, significant changes were not observed for forced expiratory flows over the midexpiratory range from before to after the 90-min FA exposure period. There were no significant correlations between the percent changes from before to after the FA exposure in FVC, FEV_1, FEV_1/FVC, $FEF_{25-75\%}$, and MVV and the 90-min average formaldehyde exposure concentrations (all $p > 0.05$; **Figure 1**).

Inflammatory Biomarkers

Circulating levels of IL-6 within the blood were unchanged from before to after the FA exposure period (Pre: 2.51 ± 6.27 pg·mL^{-1}, Post: 2.65 ± 6.32 pg·mL^{-1}; $p > 0.05$). Additionally, 15(S)-HETE did not change as a result of the FA exposure (Pre: 2547 ± 633 pg·mL^{-1}, Post: 2331 ± 728 pg·mL^{-1}; $p > 0.05$). No statistically significant relationships were observed between changes in the circulating concentrations of

IL-6 and 15(S)-HETE and any changes in pulmonary function measures including %ΔFVC (IL-6: p = 0.827; 15(s)-HETE: p = 0.787), %Δ FEV_1 (IL-6: p = 0.925; 15(s)-HETE: p = 0.067), %Δ PEF (IL-6: p = 0.591; 15(s)-HETE: p = 0.105), and %Δ $FEF_{25-75\%}$ (IL-6: p = 0.537; 15(s)-HETE: p = 0.250) or the FA exposure concentration (IL-6: p = 0.621; 15(s)-HETE: p = 0.244).

DISCUSSION

Measures of spirometry are not found to significantly change, nor are the percent changes of individuals correlated with formaldehyde concentrations during a 90-min exposure. Finally, circulating markers of inflammation were not found to change with a 90-min FA exposure, nor were they correlated with percent changes of spirometry.

Formaldehyde Exposure

Both laboratories included in the current study were well within the standards set by OSHA (**Table 2**) (ref 3). Large differences in exposure can be attributed to area of dissection with higher concentrations being the abdominal cavity and lower concentrations dissection of the back and head[11]. While the concentrations of FA did not exceed the OSHA guidelines, large variability is shown in our measurements of FA concentration in the air which would agree with studies that examined individual exposure levels to concentrations of the room [15,29].

Pulmonary Function

There were no detriments to pulmonary function associated with acute exposure to FA[30]. Attention was drawn to variables associated with effort of the participant as a non-maximal maneuver could skew the data. Observation of percent predicted values confirm high performance across variables (>80 % predicted), thus are indicative of maximal effort. The lack of difference could be explained by duration of FA exposure not being great enough to elicit a decrement to bronchial function for a decrease in FEV_1; however, the assumption of decreased bronchial function is not likely as Schachter and coauthors found that airway resistance was not altered in response to FA [31] . As previously stated, FA is readily dissolved in the upper respiratory tract resulting in a required increase ventilation or FA concentration to elicit the bronchial decrement associated with decreased FEV_1 [32]. The non-significant pre to post exposure FVC values are within reason of the current literature. In a study by Harving et al. [33], asthmatic non-smoking persons were subjected to FA exposure at differing dosages in which no change in FEV_1 was seen. Similar studies have

attempted to determine the acute effects of FA on both asthmatics and non-asthmatic persons, to which many find that there are no decrements in pulmonary function [31,34].

For pulmonary function changes to occur, lung tissue would have to undergo massive remodeling [35-37]. Remodeling of significance would likely take week to months of exposure before pulmonary function saw a true decrement [38]. An animal study which used approximately 22.5 ppm 1 hr/day, 5 days/week for two weeks saw significant increases in total cells of the bronchoalveolar lavage which could be attributed to the huge amount of formaldehyde showing that large amounts of formaldehyde would be required for an acute exposure to expose significance in formaldehyde exposure [39].

Furthermore, histamine thresholds did not change in asthmatics pre to post exposure confirming that the perceived irritation may not be associated with inflammatory markers, or at least the ones tested for [33]. A longitudinal study of medical students found that a month after baseline, there was a significant decrease in FVC and FEV_1 to which the authors suspect that the FA dissolved in the upper respiratory tract elicit an immune response[40]. Animal studies have found an increased inflammatory response based on FA concentration as it relates to the bronchoalveolar lavage fluid and oxidative stress [23,41,42].

Inflammatory Biomarkers

IL-6 is a cytokine associated with inflammation. It has been shown that increased levels of IL-6 are related to reduced FVC as well as reduced FEV_1 [43,44]. Additionally, it has been found that the amount of IL-6 is related to the concentration of pollution [45]. Due to the inflammation associated with FA exposure as well as the route of exposure via vapors, we hypothesize that IL-6 would be increased as a function of pulmonary function. While our subjects were exposed to FA for 90 minutes, it is possible that this is not a sufficient time to invoke an inflammatory response given that an ischemic stimulus was only party visible on immunopositivity scales between 4-6 hr following stimuli where as widespread expression was observed following 6-8 hr in cell cultures[46].

15(S)-HETE was chosen as a biomarker inflammation as it has shown, in cell culture, the largest increase in the presence of arachidonic acid[47]. 15(S)-HETE is a metabolite of arachidonic acid, via 15-lipoxygenase,

and a member of a class of biochemical known as eicosanoids. Epithelial cells are often the first to observe the consequences of irritable substances, such as FA, given the highest concentration will be incurred. While 15-HETE is one of many eicosanoids associated with the lungs, it has particular implications as cell cultures of epithelial cells have demonstrated the largest production of 15-HETE compared to other metabolites of the lipoxygenase pathway[47]. Indeed, high production of a biochemical is not always causative, some eicosanoids are known to cause constriction of pulmonary smooth muscle [48] as well as stimulate airway mucus secretion[49]. Due to the known irritations and inflammation associated with FA exposure, we hypothesize that 15(S)-HETE will increase in response to an acute FA exposure.

Conclusion

There are no differences between pre to post-acute exposure pulmonary function in young otherwise healthy adults. Furthermore, the percent change of FVC and FEV_1 pre to post were not correlated with the average concentration of FA inspired during laboratory cadaver dissection. While this study's findings are similar to other research, it is still unclear as to how studies have found decrements in pulmonary function following acute exposure. Potential improvement of the findings could be found if assessment of subjective symptoms were recorded via survey as seen in some of the previous literature [5,14,16,17,50]. Further research should be completed to assess whether percent changes in pulmonary function are associated with average FA exposure as this could explain occupational hazards.

Acknowledgements

This research would not be possible without the financial support of Appalachian State University and Elon University.

Conflict of Interest

No conflicts of interest, financial or otherwise, are declared by the authors.

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Tables

Table 1. Subject Characteristics

Subjects	N	Age (yr)	HT (cm)	WT (kg)	BMI ($kg \cdot m^{-2}$)
Male	2	28 ± 6	183 ± 3	84 ± 3	25 ± 2
Female	15	23 ± 2	164 ± 6	65 ± 15	24 ± 4
Combined	17	24 ± 3	166 ± 8	66 ± 15	24 ± 4

Values are expressed as mean ± SD. BMI, body mass index.

Table 2. Formaldehyde concentration (ppb)

30 min	60 min	90 min
196	231	271
253	356	225
125	113	117
130	232	231
238	248	321
320	316	232
193	473	389
238	329	362
11	29	16
10	29	10
36	33	44

Individual formaldehyde readings starting from full first 30 min of dissection.

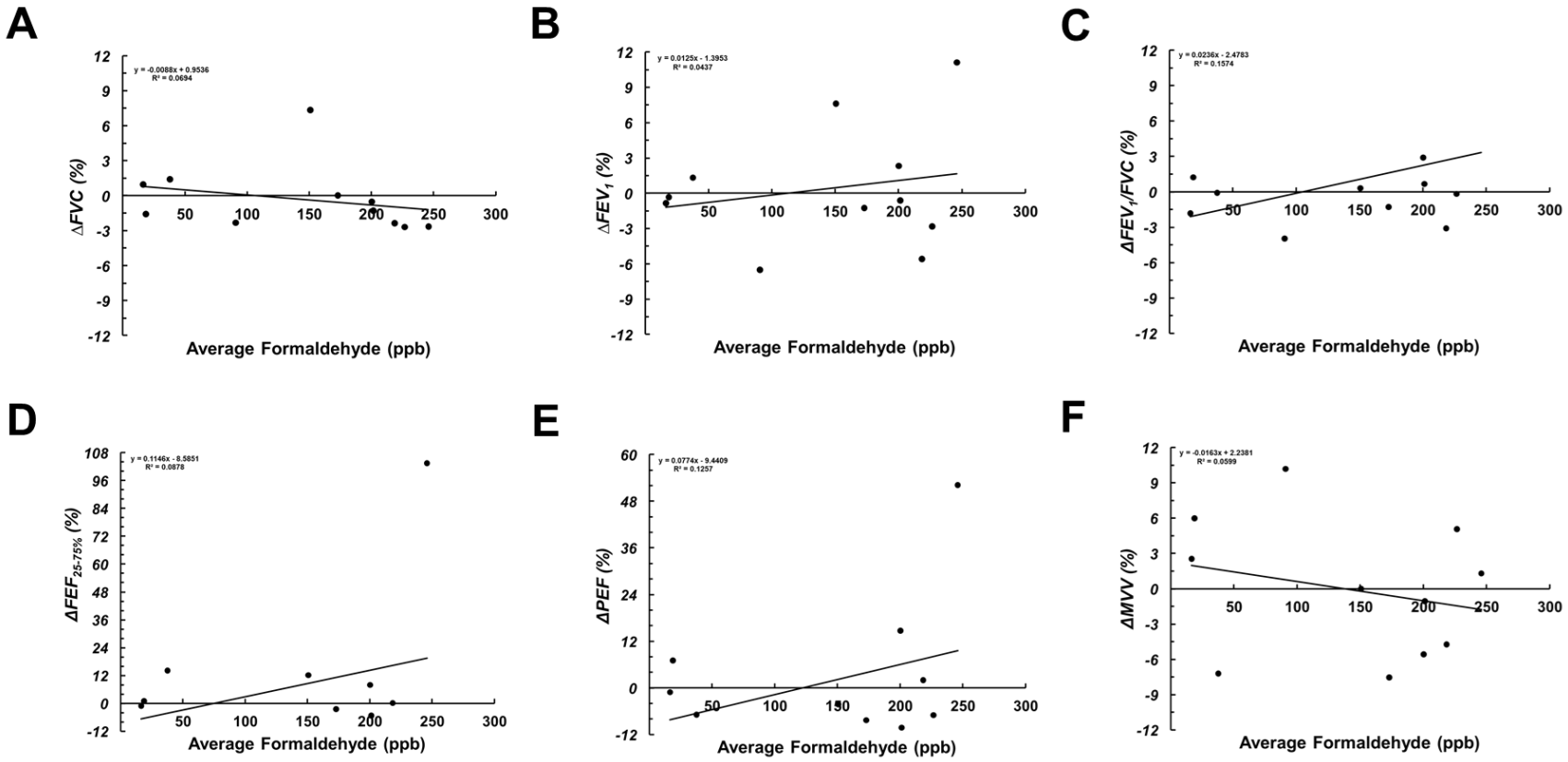
Table 3. Pulmonary Function

Measurement	Pre	Post	*p* value
FEV1/FVC, %	85.10 ± 6.58	85.40 ± 5.83	0.719
$FEF_{25-75\%}$	3.81 ± 1.09	3.86 ± 1.05	0.791
%pred	100 ± 25	101 ± 27	0.755
$FEF_{25\%}$, $L \cdot s^{-1}$	6.35 ± 1.61	6.43 ± 1.74	0.802
$FEF_{50\%}$, $L \cdot s^{-1}$	4.50 ± 1.38	4.79 ± 1.50	0.239
$FEF_{75\%}$, $L \cdot s^{-1}$	2.00 ± 0.70	1.87 ± 0.47	0.283
PEF, $L \cdot s^{-1}$	7.62 ± 1.78	7.74 ± 1.90	0.638
%pred	102 ± 13	104 ± 17	0.664
FET 100%	5.54 ± 1.53	5.32 ± 1.53	0.409
MVV, $L \cdot min^{-1}$	122 ± 27	125 ± 33	0.424
%pred	102 ± 16	103 ± 19	0.428
f_B, breaths·min^{-1}	103 ± 15	105.67 ± 18	0.471
V_T, L	1.23 ± 0.34	1.22 ± 0.30	0.751

Values are expressed as mean ± SD. All variables that have reference values in the Third National Health and Nutrition Examination Survey have been reported beneath as % predicted, respectively. FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; FEF, forced expiratory flow; PEF, peak expiratory flow; FET, forced expiratory time; MVV, maximal voluntary ventilation; fB, frequency of breathing; VT, tidal volume. * Significantly difference pre to post ($p < 0.05$)

Figure Legends:

Figure 1: The percent change in an individual's force vital capacity (FVC, A), forced expiratory volume in 1 s (FEV_1,B), FEV_1/FVC (C), midexpiratory flow of middle 50% of FVC ($FEF_{25-75\%}$, D), peak expiratory force (PEF, E), and maximal voluntary ventilation (MVV,F) were not correlated with their individual average formaldehyde exposure (ppb).


A
y = -0.0088x + 0.9536
R² = 0.0694
ΔFVC (%)
Average Formaldehyde (ppb)
B
y = 0.0125x - 1.3953
R² = 0.0437
ΔFEV1 (%)
Average Formaldehyde (ppb)
C
y = 0.0236x - 2.4783
R² = 0.1574
ΔFEV1/FVC (%)
Average Formaldehyde (ppb)
D
y = 0.1146x - 8.5851
R² = 0.0878
ΔFEF25-75% (%)
Average Formaldehyde (ppb)
E
y = 0.0774x - 9.4409
R² = 0.1257
ΔPEF (%)
Average Formaldehyde (ppb)
F
y = -0.0163x + 2.2381
R² = 0.0599
ΔMVV (%)
Average Formaldehyde (ppb)

Chapter 3

Electronic Cigarettes (EC) are a popular alternative to tobacco smoking that has taken hold since the early 2000s. This fact could be due to the reduced number of chemicals as well as reduced concentrations of EC vapor compared with tobacco smoke (44) which did include formaldehyde. Given the novelty of the EC, questions have been synthesized to assess the safety of EC use as it compares to tobacco smoking. Acutely, it has been shown that lung function is maintained with no differences between placebo, tobacco and EC trials (45). Given that pulmonary function is maintained, it is interesting that the increasing concentration of nicotine significantly decreases the expired NO thus leading to increased airway resistance (46), though this has been observed in studies not focused on the concentration of particles or nicotine (47).

Contrary to the, relatively, maintained lung function of acute EC usage, seldom are humans stationary. Therefore, studies have attempted to assess aerobic function of persons that use EC which has found that *in vitro*, EC vapor elevate mitochondrial reactive oxygen species (48).

Speculations on exercise responses to acute EC usage might be made by observing articles that measure traditional cigarettes. For instance, it was observed that increased carbon monoxide was bound to hemoglobin when smoking compared with nonsmoking and ultimately resulted in a reduced $\dot{V}O_{2max}$ (49). However, as previously stated, the use of EC is increasing and the normal activity of people should be objective data while using these products given the link of tobacco smoking to chronic, life-changing pathologies.

Title: **Metabolic and Ventilatory Responses to Exercise Following Electronic Cigarette Usage**

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Keywords: Electronic cigarette, exercise, metabolic, ventilation

Word Count: 5636

ABSTRACT

BACKGROUND: Increased airway resistance has been shown in electronic cigarette users which has important implications to cardiorespiratory function during exercise, where large expiratory air flow rates are required to meet the ventilatory and metabolic demands.

PURPOSE: To investigate the acute effects of EC use on exercise tolerance and exertional dyspnea in young adults.

METHODS: Male participants (N=10; 21±2yr, 180.4±8.1cm, 84.9±13.3kg) visited the laboratory on five occasions. Subjects completed pulmonary function testing for familiarization (FAM) purposes during the initial visit. During the subsequent two visits, subjects inhaled from an EC with (EC+) or without (EC-; i.e., placebo) the nicotine cartridge in random order. Two additional visits performed the EC smoking challenge followed by an incremental exercise test to volitional exhaustion on a cycle ergometer.

RESULTS: Upon study entry, all subjects displayed pulmonary function measurements above the lower limits of normal. Airway resistance increased comparing FAM with EC- and EC+, though no differences between EC- and EC+ were observed. Maximal oxygen consumption was not different between trials ($p > 0.05$). Ventilation, operational lung volumes, and the ventilatory equivalents for O_2 and CO_2 were only different by intensity and did not change due to the EC challenge. Heart rate was not different between conditions; however, oxygen pulse was lower in the EC+ (p=0.043).

CONCLUSION: These data suggest that individuals may experience differences to airway function and oxygen pulse suggesting unperceivable alterations at rest and during exercise. Potentially, exercise stimulus is sufficient to overcome or override the alterations to airway resistance.

Abstract word count: 247

Introduction

Electronic cigarettes (EC) are devices intended to deliver an aerosol by rapidly heating an inhaled solution containing nicotine and possible flavorings and/or additives. They are marketed as a method for quitting tobacco smoking, as a healthier alternative to tobacco smoking, and as a method to "smoke" in public spaces. Many teenagers and young adults, however, are increasingly using the unregulated devices due the belief that EC are less harmful than tobacco cigarettes [1] and/or the appeal of candy and fruit flavors. As of 2018 estimates suggest that as many as 25.8% of US adults aged 18-24 have used an EC and nearly 8% of whom are current users[2]. While there is much ongoing debate about their benefits and efficacy as a tobacco cessation aid [3-5], little is known about the immediate health consequences posed by their use.

Assessments of respiratory function with EC use have been conducted to elucidate its potential effects on measurements of air flow, volume, and gas exchange[6-8]. Six months of daily EC usage has been associated with decrements to forced expiratory volume in 1 s (FEV_1), the ratio of FEV_1 to forced vital capacity (FVC) and forced expiratory flow in the middle 50% of FVC ($FEF_{25-75\%}$)[9]. Additionally, chronic EC users have been found to exhibit a greater degree of ventilation-perfusion mismatch when compared with non-EC users [10]. In contrast, in naïve subjects, pulmonary function does not appear to be altered with acute EC use, as FEV_1, FVC, and $FEF_{25-75\%}$ are conserved [6-8]. Yet, it is recognized airway function can be impaired prior to observing significant changes in common pulmonary function measurements. Impulse oscillometry, a more sensitive technique to examine airway function, has demonstrated that EC usage increases airway resistance despite not affecting spirometry measurements [6,7] Furthermore, the fraction of expired nitric oxide (FE_{NO}), an indicator of bronchial function, is altered following acute EC usage [6,11]. These findings suggest that airway function is impaired, even when no decrements to common indicators of spirometry are observed, and that over time, gas exchange abnormalities may result in chronic users. These potential perturbations may be exaggerated and alter ventilatory responses during exercise when high levels of airflow and ventilation are required.

The link between cardiovascular health and tobacco cigarette usage is indisputable. Tobacco smokers are at greater risk of developing cardiovascular diseases, including hypertension, peripheral artery disease, and myocardial infarction. Yet, it is unclear to what extent acute and chronic EC usage affects cardiovascular function. Recently, it was demonstrated that EC usage increased heart rate (HR), pulse wave velocity, and augmentation index corrected to 75 heart beats per minute measured at rest which are indicative of potential future events given a trend to tachycardia as well as stiffening of the vessels[7,12,13]. These potential risk factors are not maintained when myocardial function was observed in chronic EC users suggesting that while vascular health is at risk, myocardial function could be preserved[14].. Given that high temperature vaporization of the EC liquid has shown to produce formaldehyde, decrements to cardiovascular function may result from the formaldehyde exposure as our group has shown arterial stiffening with acute exposure to formaldehyde in addition to decrements to flow-mediated dilation and increased heart rate[15,16]. The potential for formaldehyde to be the cause of cardiovascular detriments associated with EC is not conserved across all studies as flow-mediated dilation has also shown to be preserved when comparing EC users, tobacco cigarette users, and naïve subjects[17].

The potential physiologic consequences to acute EC usage have important implications to the respiratory and cardiovascular function exhibited during exercise, where large expiratory air flow rates and increased blood flow to active tissues are required to meet the ventilatory and metabolic demands. Furthermore, in the face of mechanical constraints to ventilation during exercise, which can occur with increased airway resistance, breathing may be forced to occur at higher lung volumes closer to total lung capacity where maximal expiratory flows are greater [18]. However, this shift in the operational lung volume occurs where the mechanical work and metabolic cost of breathing are greater. Subsequently, the perception of dyspnea during exercise may be elevated as a result of the increased respiratory muscle effort required to achieve the necessary exercise ventilation and may serve as a limitation to exercise performance [19]. Yet, it is unknown whether… Thus, the purpose of this study was to investigate the acute effects of EC use on cardiorespiratory function and exertional dyspnea during incremental exercise in young adults. We

hypothesized that cardiorespiratory function would be altered, and exertional dyspnea increased in young adults following EC use.

Methods

Subject Characteristics

Ten (N=10) male subjects participated in the current investigation. Subjects were otherwise healthy and free of any known metabolic, cardiovascular, and respiratory disease. Additionally, five subjects reported cessation from smoking and history of less than 2 pack years and no longer habitually smoking. Subjects were not currently aerobically training as reported by an International Physical Activity Questionnaire (IPAQ). All subjects gave written informed consent to protocols approved by the Institutional Review Board of Appalachian State University. The study was performed in accordance with the ethical standards as described in the Declaration of Helsinki.

Protocol

Subjects visited the laboratory on five occasions, each separated by a minimum of 48 h, to complete all testing procedures. Following completion of informed consent and medical history questionnaires, subjects completed a pulmonary function familiarization test (FAM). Subsequently, subjects were familiarized to the EC smoking task. During visits two and three, subjects performed the EC smoking task with (EC+) and without (EC-; i.e., placebo) the nicotine cartridge in random order before completing pulmonary function testing. Similarly, during visits four and five, subjects performed the EC smoking task with (EC+) and without (EC-; i.e., placebo) the nicotine cartridge in random order before completing an incremental exercise test to volitional exhaustion.

EC Smoking Task

A commercially available EC was chosen without bias (blu™ Magnificent Menthol). The nicotine content of the menthol flavored cartridge was 2.4% (24 mg/mL) accompanied with glycerin vehicle (75%). The product was a classic EC (first generation) with a rechargeable cartomizer. This particular device has been shown to elicit the largest on plasma nicotine when compared with other EC of similar styles [20]. Subjects inspired from an adapter connected to the EC every 30 s for 10 min for a total of 20 inspirations from the EC. The adapter contained a side port for the measurement of mouth pressure. Subjects were instructed to generate an inspiratory mouth pressure (P_i) of -20 cmH_2O for 2 s, as this was meant to simulate typical behavior when smoking[11]. Subjects received continuous visual feedback displayed on a computer monitor regarding the achieved and target mouth pressures (DASYLab, Norton, MA). All expired air was collected into an opaque Douglas bag connected to a one-way valve. The EC smoking task was designed in an effort to blind subjects to the nicotine containing cartridge.

Pulmonary Function

Spirometry and lung volumes were measured according to American Thoracic Society guidelines[21]. Measurements of spirometry were conducted in a volume-displacement plethysmograph (Vmax, CareFusion, Yorba Linda, CA) to account for gas compression artifact. Subjects were asked to maximally expire following a full inspiration. The occluded panting technique was used to measure functional residual capacity (FRC), which was followed by an inspiratory capacity and complete expiratory maneuvers to measure total lung capacity (TLC) and residual volume (RV), respectively. Airway resistance was measured using the changes in flow and mouth pressures during a panting maneuver with an open shutter. Triplicate measurements of spirometry (FVC <150 mL), lung volumes (FRC $\leq$ 5%), and airway resistance were measured. Volumes were corrected to body temperature, pressure, and saturated conditions. Absolute

values are reported as well as percent of predicted (%pred) using the third National Health and Nutrition Examination Survey (NHANES III) prediction equations[22].

Incremental Exercise Test

An exercise test was performed on an upright electromagnetically braked cycle ergometer (Lode Corival, Netherlands). Subjects were instrumented with a forehead pulse oximeter (Nellcor N-595, Minneapolis, MN) and a heart rate strap (Polar Electro, Kempele, Finland). Metabolic gases were analyzed using a metabolic cart (Parvo Medics, Salt-Lake City, Utah).

Subjects remained seated on the cycle ergometer for six minutes for the collection of resting measurements. Two inspiratory capacity (IC) maneuvers were completed during the last minute of rest to correct for expiratory drift for analysis of breathing mechanics. Following the IC maneuvers, subjects began cycling at 30 W using a cadence of 60-80 rpm. The cycling work rate increased by 30 W every minute until volitional exhaustion. During each minute of exercise, subjects completed an IC maneuver and were asked to provide ratings of perceived breathlessness (RPB; Borg scale 0-10), perceived unpleasantness of breathing (RPU; Borg scale 0-10), and perceived exertion (RPE; Borg scale 6-20). Following completion of exercise, subjects were asked to rate their affective dimensions of dyspnea (unpleasantness, anxiety, depression, anger, frustration, and fear) on a visual analog scale (VAS) pertaining to their highest achieved RPB during exercise[23].

Flow, volume, and pressure

Air flow was measured using pneumotachographs (Model 4813, Hans Rudolph, Kansas City, MO) on both the inspiratory and expiratory sides of a low resistance two-way valve (model 2700, Hans Rudolph, Kansas City, MO). The pneumotachograph located on the expiratory side was heated to minimize condensation.

Inspiratory and expiratory air flows were integrated to yield volumes. Pressures were measured at the mouth (P_m) via calibrated pressure transducer (+30 cmH_2O; DP45, Vaildyne, Northridge, CA). All raw data were continuously collected using Spike 2 software (Cambridge Electrical Design, United Kingdom) on a Micro1401 32-bit AD converter (Cambridge Electrical Design, United Kingdom). Mouth pressures were integrated by time and multiplied by breathing frequency to achieve an index of inspiratory and expiratory work of breathing (WoB) as outlined by Collett, 1985[24].

Operational lung volumes

Approximately 8-12 breaths were analyzed during each stage to examine operational lung volumes. End-expiratory lung volumes (EELV) were calculated as TLC minus IC. Tidal volume (V_T) was added to EELV to calculate the end-inspiratory volumes (EILV). Expiratory flow limitation (EFL) was calculated by overlaying the exercise tidal flow-volume loop with the compression-free maximal flow-volume loop (mFVL) obtained during spirometry. The percentage of EFL was calculated as:

$$\text{EFL } \% = \left(\frac{\text{volume overlap}}{V_T}\right) * 100$$

Statistical Analysis

Analysis of findings were assessed using commercially available software (IBM SPSS Statistics Version 27, Armonk, NY). A repeated measure analysis of variance (ANOVA) was used to compare parameters measured during the EC smoking task across the four intervention trials. Additionally, a repeated measures ANOVA was performed to examine the potential difference in pulmonary function between FAM, EC-, and EC+. Two-way repeated measures ANOVA (condition by stage) were performed to assess cardiorespiratory responses throughout exercise during EC- and EC+. Violations of Mauchly's Test of Sphericity were accommodated using the Huynh-Feldt correction. Post hoc analyses using a least

significant difference (LSD) were performed where family-wise significance was detected. Statistical significance was set at $p \leq 0.05$. Data are expressed as mean ± standard deviation (SD). Partial eta squared (η^2_p) was used to determine effect size (small effect: $\eta^2_p = 0.01$; medium effect: $\eta^2_p = 0.06$; large effect: $\eta^2_p = 0.14$).

Results

Subject characteristics are displayed in **Table 1**.

EC Smoking Task

Subject's peak P_m was not different between conditions for the EC smoking task (EC-: -20.34 ± 3.95 cmH_2O; EC+: -21.30 ± 2.21 cmH_2O; p = 0.380, $\eta^2_p = 0.111$). Additionally, a one second moving average of P_m was not different between conditions for the EC smoking task (EC-: -19.95 ± 3.94 cmH_2O; EC+: -20.95 ± 1.85 cmH_2O; p = 0.390, $\eta^2_p = 0.107$). The integrated P_m was not different between EC smoking tasks (EC-: -25.56 ± 8.32 $cmH_2O \cdot s$; EC+: -28.24 ± 5.01 $cmH_2O \cdot s$; p = 0.295, $\eta^2_p = 0.155$).

Pulmonary Function

With the exception of FEV_1 expressed as a percent of predicted, spirometry and lung volume measurements were similar between FAM, EC-, and EC+ trials (**Table 2**). MVV was not different between the three trials (99 ± 10 %pred, 101 ± 10 %pred, 101 ± 8 %pred, respectively; $p > 0.05$). Measurements of airway resistance (Raw: 1.75 ±0.65 $cmH_2O \cdot s \cdot L^{-1}$, 2.05 ± 0.62 $cmH_2O \cdot s \cdot L^{-1}$, and 1.98 ± 0.53 $cmH_2O \cdot s \cdot L^{-1}$; $p = 0.039$, $\eta^2_p = 0.303$) were significantly greater following the EC smoking task than during FAM. Yet, airway conductance (Gaw: 0.63 ± 0.20 $L \cdot cmH_2O^{-1} \cdot s^{-1}$, 0.53 ± 0.12 $L \cdot cmH_2O^{-1} \cdot s^{-1}$, 0.54 ± 0.11 $L \cdot cmH_2O^{-1} \cdot s^{-1}$; $p = 0.079$, $\eta^2_p = 0.290$) was not different between trials. Raw and Gaw were not different between EC- and EC+ ($p > 0.05$). Additionally, specific airway resistance (sRaw) was significantly greater following the EC smoking tasks than during FAM (FAM: 7.18 ± 1.60 $cmH_2O \cdot s$; EC-:

8.65 ± 2.09 $cmH_2O \cdot s$; EC+: 8.41 ± 1.77 $cmH_2O \cdot s$; $p = 0.008$, $\eta^2_p = 0.415$). Significant decreases of specific airway conductance (sGaw) were observed following EC smoking task compared to FAM (FAM: 0.146 ± 0.03 $cmH_2O^{-1} \cdot s^{-1}$; EC-: 0.123 ± 0.03 $cmH_2O^{-1} \cdot s^{-1}$; EC+: 0.125 ± 0.03 $cmH_2O^{-1} \cdot s^{-1}$; $p = 0.025$, $\eta^2_p = 0.390$)

Cardiometabolic Responses to Exercise

Oxygen consumption ($\dot{V}O_2$) and carbon dioxide production ($\dot{V}CO_2$) increased from rest to peak exercise ($p < 0.05$, $\eta^2_p = 0.963$; **Figure 1**). $\dot{V}O_{2peak}$ (EC-: 3.11 ± 0.42 $L \cdot min^{-1}$; EC+: 3.15 ± 0.56 $L \cdot min^{-1}$; $p = 0.655$), as well as peak power (EC-: 261 ± 38 W; EC+: 270 ± 40 W; $p = 0.081$), were not different between conditions. However, time to exhaustion was greater in the EC+ condition compared with EC- (EC-: 9.66 ± 1.35 min; EC+: 9.97 ± 1.21 min; $p = 0.036$). When expressed as a percent of peak, $\dot{V}O_2$ (EC-: 49 ± 2 %; EC+: 47 ± 2 %; $p = 0.014$, $\eta^2_p = 0.510$) was significantly different between conditions; however, absolute $\dot{V}O_2$ (EC-: 1.51 ± 0.04 $L \cdot min^{-1}$; EC+: 1.44 ± 0.05 $L \cdot min^{-1}$; $p = 0.172$, $\eta^2_p = 0.196$) and relative $\dot{V}O_2$ (EC-: 17.82 ± 0.71 $mL \cdot kg^{-1} \cdot min^{-1}$; EC+: 17.49 ± 0.599 $mL \cdot kg^{-1} \cdot min^{-1}$; $p = 0.524$, $\eta^2_p = 0.047$) were not different between conditions. A condition by intensity interaction for relative $\dot{V}O_2$ was detected ($p = 0.050$, $\eta^2_p = 0.150$); however, post hoc analysis of these data did not elucidate where the differences existed. There was no condition by intensity interaction of absolute oxygen consumed ($p = 0.165$, $\eta^2_p = 0.159$) or oxygen consumption as a percent of peak ($p = 0.098$, $\eta^2_p = 0.169$). While $\dot{V}CO_2$ change with intensity ($p < 0.001$, $\eta^2_p = 0.960$), there was no difference of condition (EC-: 1.52 ± 0.05 $L \cdot min^{-1}$; EC+: 1.45 ± 0.06 $L \cdot min^{-1}$; $p = 0.314$, $\eta^2_p = 0.112$) or of condition by intensity ($p = 0.127$, $\eta^2_p = 0.186$).

Heart rate increased with exercise intensity ($p < 0.001$, $\eta^2_p = 0.950$), yet no differences were observed between EC- and EC+ (EC-: 121 ± 4 $beats \cdot min^{-1}$; EC+: 122 ± 5 $beats \cdot min^{-1}$; $p = 0.450$, $\eta^2_p = 0.065$) or a condition by intensity interaction ($p = 0.779$, $\eta^2_p = 0.059$; **Figure 2**). O_2 pulse also increased throughout the incremental exercise ($p < 0.001$, $\eta^2_p = 0.950$). While O_2 pulse was greater throughout the incremental

exercise test during EC- compared with EC+ (EC-: 11.68 ± 0.40 mL·beats^{-1}; EC+: 11.03 ± 0.44 mL·beats^{-1}; $p = 0.043$, $\eta^2_p = 0.381$), a condition by intensity interaction was not observed ($p = 0.157$, $\eta^2_p = 0.150$).

Ventilatory Responses to Exercise

$\dot{V}_E$, V_T, and f_B increased with intensity ($p < 0.001$, $\eta^2_p > 0.9$). However, $\dot{V}_E$ and breathing pattern were not different between EC- and EC+ ($\dot{V}_E$: $p = 0.059$, $\eta^2_p = 0.289$; V_T: $p = 0.627$, $\eta^2_p = 0.077$; f_B: $p = 0.110$, $\eta^2_p = 0.192$; **Figure 3**). The duration of inspiration (Ti), duration of expiration (Te), and the ratio of Ti to the total duration of a breath (Ti/Ttot) were all found to change with increasing intensity ($p < 0.001$; **Figure 4**); however, no differences were observed between conditions ($p > 0.100$, $\eta^2_p > 0.020$) or a condition by intensity interaction ($p > 0.100$, $\eta^2_p > 0.080$).

Estimates of inspiratory and expiratory WoB during each stage were found to increase in accordance with increased ventilatory demand ($p < 0.001$, $\eta^2_p > 0.900$; **Figure 4**). Both estimates of WoB were not different between conditions ($p > 0.800$, $\eta^2_p < 0.050$) or a condition by intensity interaction ($p > 0.600$, $\eta^2_p < 0.040$).

Operational lung volumes

EELV decreased significantly with increased demand of ventilation ($p < 0.001$) while EILV simultaneously increased ($p < 0.001$). No effect of condition was observed in operational lung volumes (EILV: $p = 0.724$, $\eta^2_p = 0.016$; EELV: $p = 0.103$, $\eta^2_p = 0.298$). Further, no interaction of condition by intensity was observed in operational lung volumes ($p > 0.6$, $\eta^2_p < 0.08$). EFL was not found to be different between conditions (EC-: 40 ± 21; EC+: 34 ± 29; $p = 0.600$) and limitations were not observed prior to subject's peak exercise stage.

Perceptual Responses to Exercise

RPB ($p = 0.213$, $\eta^2_p = 0.166$), RPU ($p = 0.158$, $\eta^2_p = 0.209$), and RPE ($p = 0.582$, $\eta^2_p = 0.035$) were not different by condition, nor was a condition by intensity interaction found to be significant (RPB: $p = 0.536$, $\eta^2_p = 0.066$; RPU: $p = 0.490$, $\eta^2_p = 0.082$; RPE: $p = 0.400$, $\eta^2_p = 0.103$; **Figure 6**). Following volitional

exhaustion, a visual analog scale had subject rate their emotional responses pertaining to their peak RPB during exercise (EC- peak RPB = 6.8 ± 1.5, EC+ peak RPB = 7.1 ± 1.6). No differences were found between conditions when comparing VAS measurements of unpleasantness ($p = 0.124$, $\eta^2_p = 0.242$), anxiety ($p = 0.571$, $\eta^2_p = 0.037$), depression ($p = 0.647$, $\eta^2_p = 0.024$), anger ($p = 0.377$, $\eta^2_p = 0.088$), frustration ($p = 0.424$, $\eta^2_p = 0.072$), fear ($p = 0.480$, $\eta^2_p = 0.057$; **Figure 6**)

Discussion

The findings of this experiment demonstrate that acute usage of a first generation EC has minimal influence on pulmonary function and a minimal effect on cardiorespiratory and metabolic responses during exercise, as suggested by differences in relative $\dot{V}O_2$ and O_2pulse, as surrogate of stroke volume[25]. Given the lack of differences between the EC- and EC+ trials, these data suggest that, during incremental exercise, the introduction of EC vapors does not elicit a significant effect on the oxidative capabilities of cells, nor does EC elicit significant alterations to ventilatory dynamics.

Pulmonary function

Decrements to pulmonary function were not observed in response to the acute EC exposure which agrees with previous literature[6-8]. FVC was different between FAM and the EC smoking task trials (EC- and EC+) and not between EC- and EC+. The significant difference between FAM and the EC challenges, either EC- or EC+, seem to suggest that the inspiratory maneuver (i.e., EC smoking task inspiratory maneuver) was the stimulus that altered subjects as compared to FAM. Furthermore, lung volumes were maintained regardless of trial which would agree with previous findings.

Additionally, measurements of airway resistance and specific airway resistance were found to be significantly different between the FAM and EC smoking task trials (EC- and EC+), which suggests that the task itself, and not the EC vapors, was sufficient to alter airway resistance. Indeed, these data are consistent with previous literature that measured resistance in EC users [6,7]. Interestingly, our subjects were all males while other EC studies include females, who anatomically have increased resistance due to

decreased airway diameter, in airway resistance measures[6,7]. Therefore, our findings agree that Raw, sRaw, and Gaw are affected following EC smoking task; however, our absolute values are only indicative of male reactions to EC stimulus as females were not a part of the cohort. A study that used both males and females concluded that airway resistance at low frequency impulse oscillometry, or the larger airways, was affected to a greater extent in a EC smoking trial comparing EC+ to EC-; however, the EC- trial did not elicit the same degree of decrement and was found to not change between pre and post EC- trial[6]. This does not support the findings of our study as both EC- and EC+ were different from the FAM trial. Additionally, we did not observe differences between EC- and EC+. Indeed, impulse oscillometry is a more advanced measurement than that of the current study and must be carefully considered when comparing airway resistance.

Incremental Exercise Test

It must be acknowledged that there was no familiarization with the peak exercise test, therefore, main effect of condition would only be measured between EC- and EC+. As reported, a condition by intensity interaction was observed for relative $\dot{V}O_2$. These data agree with general trends of oxygen consumption as that of smokers versus nonsmokers with non-smokers having a slightly increased oxygen consumption at submaximal work rates[26]. While relative oxygen consumption was significant, the lack of significance in absolute $\dot{V}O_2$ is troubling as this points to subtle changes or either mass and/or O_2 consumption.

Measurements of metabolic gases revealed that O_2 consumption as a percent of $\dot{V}O_{2peak}$ were different depending on the EC smoking task (i.e., EC- versus EC+). When compared with the acute effects of traditional cigarettes, similar trends were observed where O_2 pulse was reduced while $\dot{V}O_2$ was maintained[26,27]. Additionally, no changes were noted for $\dot{V}CO_2$ between conditions, which is comparable to similar studies previously performed using traditional cigarettes[26]. The lack of differences in $\dot{V}CO_2$ does agree with the lack of differences in absolute $\dot{V}O_2$, as differences in one would suggest alterations to the other given the relationship of the gases during an incremental exercise test.

Interestingly, HR did not have a significant difference between conditions at any relative or absolute work rate. These data are contradictory to Yan and D'Ruiz who compared varying EC and observed an increased resting HR[20,28]. Consequently, though the HR was not significantly different, the O_2 pulse was decreased in the EC+ challenge condition compared with EC- suggesting that stroke volume could be influenced during acute EC exposure. These data are conflicting with traditional tobacco cigarettes as these have been shown to increase cardiac output, stroke volume, and heart rate[29]. Potential differences in findings between studies can be attributed to differences in technique (e.g., measured vs estimated) and chemical composition. Nicotine concentrations of traditional tobacco cigarettes have a range of 7.17-28.86 mg[30], and our subjects did not receive the full 24 mg of nicotine as each subject only performed 20 inspiratory doses of the EC.

Operational lung volumes, when expressed as EELV and EILV absolute or relative to TLC, were conserved regardless of challenge. Further, the timing components of breathing were not different between the EC- and EC+ trials. These findings suggest that after performing the EC smoking task, individuals will exhibit similar breathing patterns, as well as breathing mechanics. Given these results, it would be beneficial to include a FAM trial to the exercise as this would elucidate if the EC smoking challenge was the stimulus for altered breathing mechanics, or if mechanics are conserved regardless of the increased Raw, sRaw, and decreased sGaw.

Finally, no differences between conditions and no condition by intensity interaction of RPB, RPU, or RPE suggest that the EC smoking task did no invoke conscious alterations to perception. Yet, our group of subjects included previous smokers. The study design eliminated any potential alterations given the repeated measures design as separate groups could have skewed the findings since current and past smokers experience greater frequency of dyspnea symptoms[31]. Additionally, no differences in the affective dimension of dyspnea which tends to be conserved across studies unless a severe pathological state, such as chronic obstructive pulmonary disease or obesity, is present in subjects[32,33].

Conclusion

These findings are among the first to examine the acute exposure to electronic cigarettes and the influence, and lack thereof, on incremental exercise. While previous literature has assessed the pulmonary function post EC exposure, our study has elucidated that the increased airway resistance experienced following an EC exposure could be due to the inspiratory challenge. Additionally, we find that there are no alterations to exercise capacity, as expressed by peak work rate, or to the aerobic capacity of individuals following acute EC usage. Finally, the lack of differences in operational lung volumes, breathing mechanics, breathing pattern, and estimates of the work of breathing suggest that there are no immediate effects of EC use on the respiratory system in young, otherwise healthy males.

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Table 1.

Table 1. *Subject characteristics (n=10)*	
Height, cm	182.4 ± 9.7
Weight, kg	83.9 ± 13.2
BMI, $kg \cdot m^{-2}$	25.2 ± 3.6
IPAQ, $MET \cdot min \cdot week^{-1}$	3474 ± 2196
Smoking history, $packs \cdot yr^{-1}$	0.13 ± 0.44

BMI, body mass index; IPAQ, International Physical Activity Questionnaire. Measurements reported are from the familiarization visit. Measurements of height were kept constant across visits. Weight and BMI were not different between visits ($p > 0.05$). Smoking history includes all subjects; however, only 5 subjects reported having a history of smoking.

Table 2.

Table 2. *Pulmonary Function (n=10)*

	FAM		EC-		EC+			
	Value	%Pred	Value	%Pred	Value	%Pred	*p* value	η^2_p
$FEV_{1,}$ L	5.05 ± 0.75	104 ± 7	4.96 ± 0.79	102 ± 7	4.90 ± 0.76*	101 ± 9*	0.111	0.217
FVC, L	6.27 ± 0.83	107 ± 5	6.10 ± 0.92	104 ± 5	6.07 ± 0.86	104 ± 5	0.010	0.399
FEV_1/FVC, %	81 ± 5	94 ± 6	81 ± 6	95 ± 6	81 ± 6	94 ± 7	0.689	0.040
PEF, $L{\cdot}s^{-1}$	11.09 ± 1.42	107 ± 10	10.81 ± 1.05	104 ± 9	10.45 ± 0.89	101 ± 8	0.133	0.224
TLC, L	7.62 ± 1.36	101 ± 11	7.74 ± 1.15	103 ± 8	7.59 ± 1.28	101 ± 10	0.320	0.119
FRC, L	3.96 ± 0.84	117 ± 20	3.92 ± 0.92	116 ± 23	3.74 ± 0.87	111 ± 22	0.270	0.135
FRC/TLC, %	52 ± 8	109 ± 17	50 ± 7	104 ± 13	49 ± 7	102 ± 13	0.410	0.094
RV, L	1.36 ± 0.68	83 ± 39	1.64 ± 0.62	101 ± 36	1.43 ± 0.68	88 ± 40	0.090	0.235
RV/TLC, %	17 ± 7		21 ± 7		18 ± 7		0.141	0.196

FAM, familiarization; EC-, placebo EC smoking task; EC+, electronic cigarette EC smoking task; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; PEF, peak expiratory force; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; η^2_p, partial eta squared. * significantly different from FAM. *p* values are univariate comparisons of the values, not % pred, andη^2_p is the effect size of the comparison.

Figure 1. Placebo condition (EC-) and experimental condition (EC+) are represented as black squares and circles, respectively. Volume of oxygen consumed ($\dot{V}O_2$; A) versus stage was not different between condition ($p = 0.172$). Produce volume of carbon dioxide ($\dot{V}CO_2$; B) was not found to be significantly different between conditions ($p = 0.314$). $\dot{V}CO_2$ relative to $\dot{V}O_2$, or respiratory exchange ratio, was not different between conditions ($p = 0.575$).

Figure 2. Placebo condition (EC-) and experimental condition (EC+) are represented as black squares and circles, respectively. Heart rate (A) was not different between conditions ($p = 0.450$). Additionally, the oxygen pulse (O2 pulse; B) was lower in the EC+ challenge ($p = 0.043$).

Figure 3. Placebo condition (EC-) and experimental condition (EC+) are represented as black squares and circles, respectively. Tidal volume (V_T; A) was not different between conditions ($p = 0.588$). Breathing frequency (fB; B) was not different between conditions ($p = 0.598$). Ventilation (V_E; C) was not different between conditions ($p = 0.951$). Ventilatory efficiency (D), expressed as ventilation relative to expired volume of carbon dioxide (V_E/VCO_2), was not found to be different between conditions ($p = 0.331$).

Figure 4: Placebo condition (EC-) and experimental condition (EC+) are represented as black squares and circles, respectively. Maximal mouth pressures were measured during inspiration (P_i; A) and expiration (P_e; B) and were not different between conditions (P_i: $p = 0.749$; P_e: $p = 0.453$). The duty cycle of breathing relative to total breath duration (T_i/T_{Total}; C) was not different between conditions ($p = 0.683$). Expired duration (T_e; D) was not different between conditions ($p = 0.181$). Rest consisted of N = 9 while exercise consisted of the entire sample (N = 10).

Figure 5: Placebo condition (EC-) and experimental condition (EC+) are represented as squares and circles, respectively. End-inspiratory and end-expiratory volumes (EILV and EELV, respectively) are shown versus stage of the test (A) and versus ventilation (B) where black markers are EELV and white markers are EILV. Neither EILV nor EELV were significantly different between conditions ($p = 0.244$ and $p = 0.864$, respectively). Rest consisted of N = 9 while exercise consisted of the entire sample (N = 10).

Figure 6: Placebo condition (EC-) and experimental condition (EC+) are represented as black squares and circles, respectively. During exercise, ratings of perceived breathlessness (RPB; A), unpleasantness (RPU; B), and exertion (RPE; C) were recorded. A visual analog scale (D) was used to assess unpleasantness of breathing, depression, anxiety, frustration, anger, and fear associated with peak ratings of perceived breathlessness. No differences were observed between conditions of RPB, RPU, or RPE ($p = 0.213$, $p = 0.158$, and $p = 0.102$, respectively). Visual analog scales were not different between condition (unpleasantness, $p = 0.124$; anxiety, $p = 0.571$; depression, $p = 0.647$; anger, $p = 0.377$; frustration, $p = 0.424$; fear, $p = 0.480$).

Figure 1.

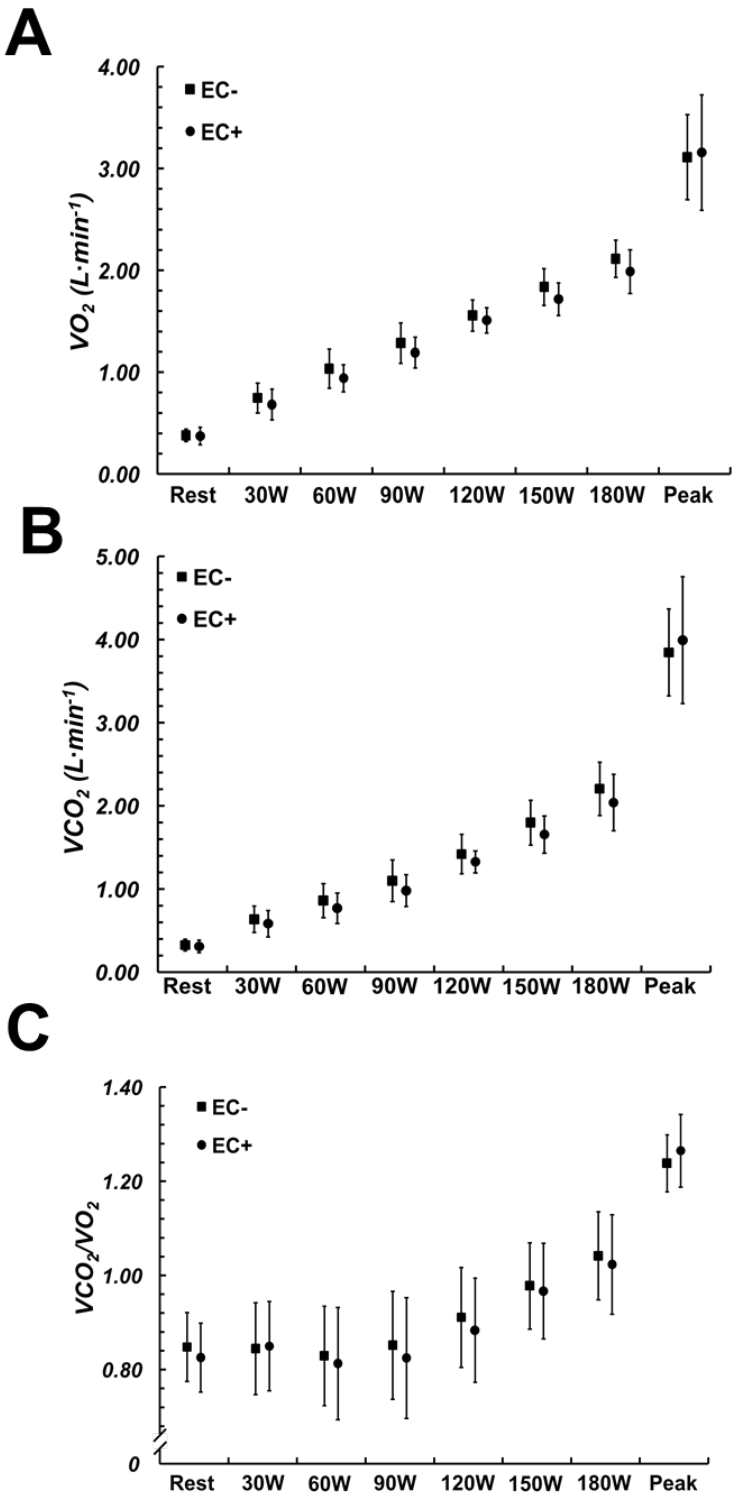

A
4.00
3.00
2.00
1.00
0.00
EC-
EC+
VO2 (L·min-1)
Rest
30W
60W
90W
120W
150W
180W
Peak
B
5.00
4.00
3.00
2.00
1.00
0.00
EC-
EC+
VCO2 (L·min-1)
Rest
30W
60W
90W
120W
150W
180W
Peak
C
1.40
1.20
1.00
0.80
0
EC-
EC+
VCO2/VO2
Rest
30W
60W
90W
120W
150W
180W
Peak

Figure 2

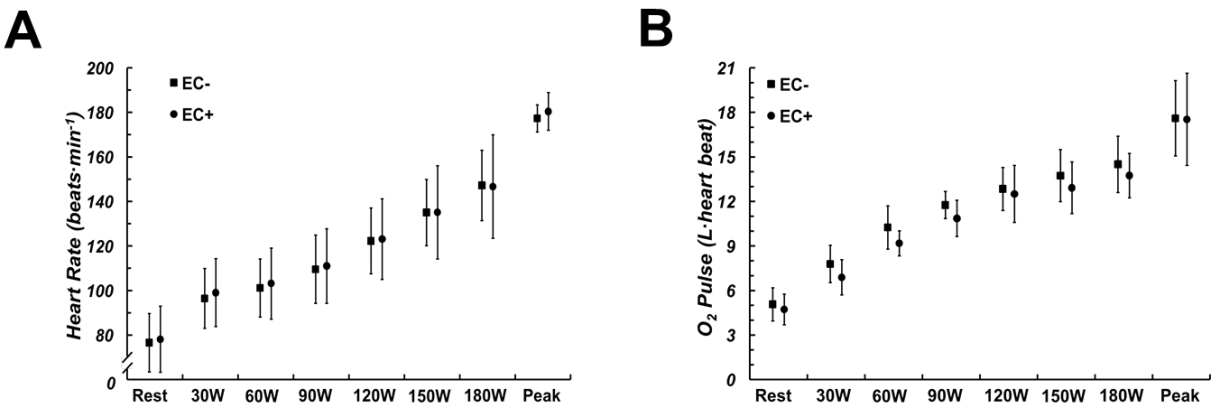

A
B
EC-
EC+
Heart Rate (beats·min-1)
O2 Pulse (L·heart beat)
Rest
30W
60W
90W
120W
150W
180W
Peak

Figure 3.

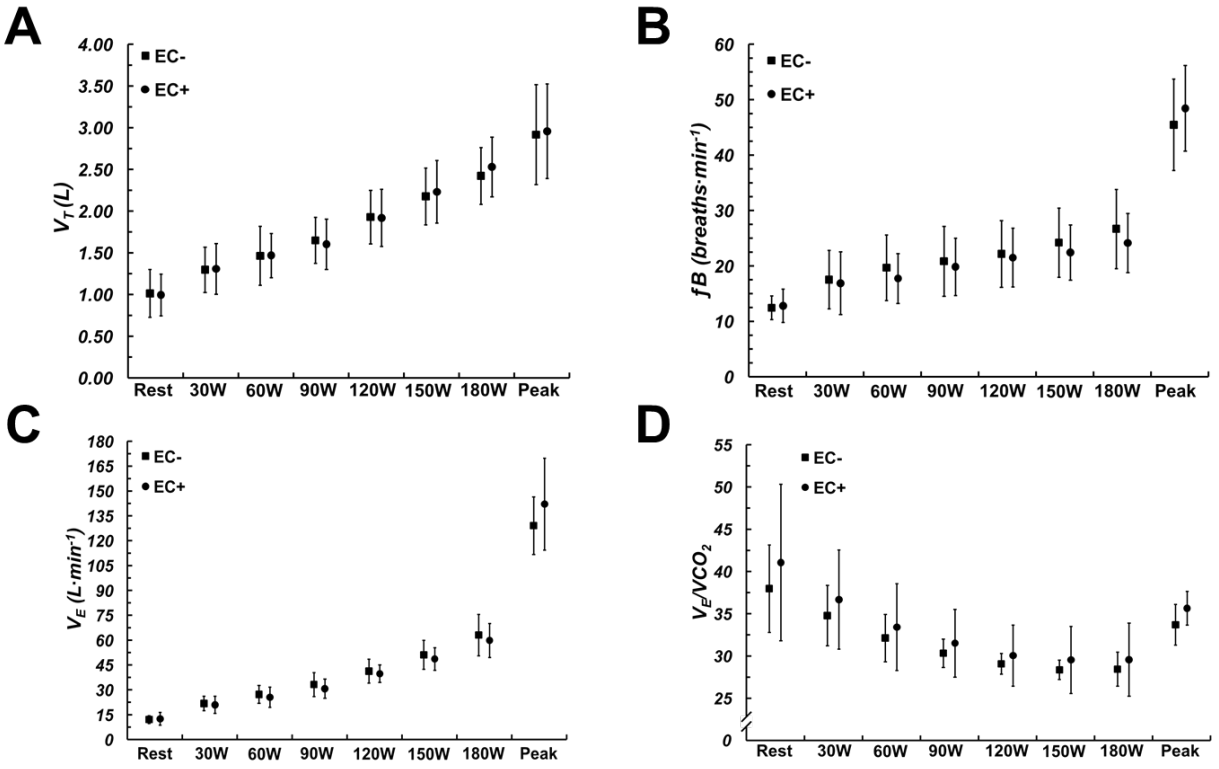

A
B
C
D
EC-
EC+
V_T(L)
fB (breaths·min^{-1})
V_E (L·min^{-1})
V_E/VCO_2
Rest
30W
60W
90W
120W
150W
180W
Peak

Figure 4.

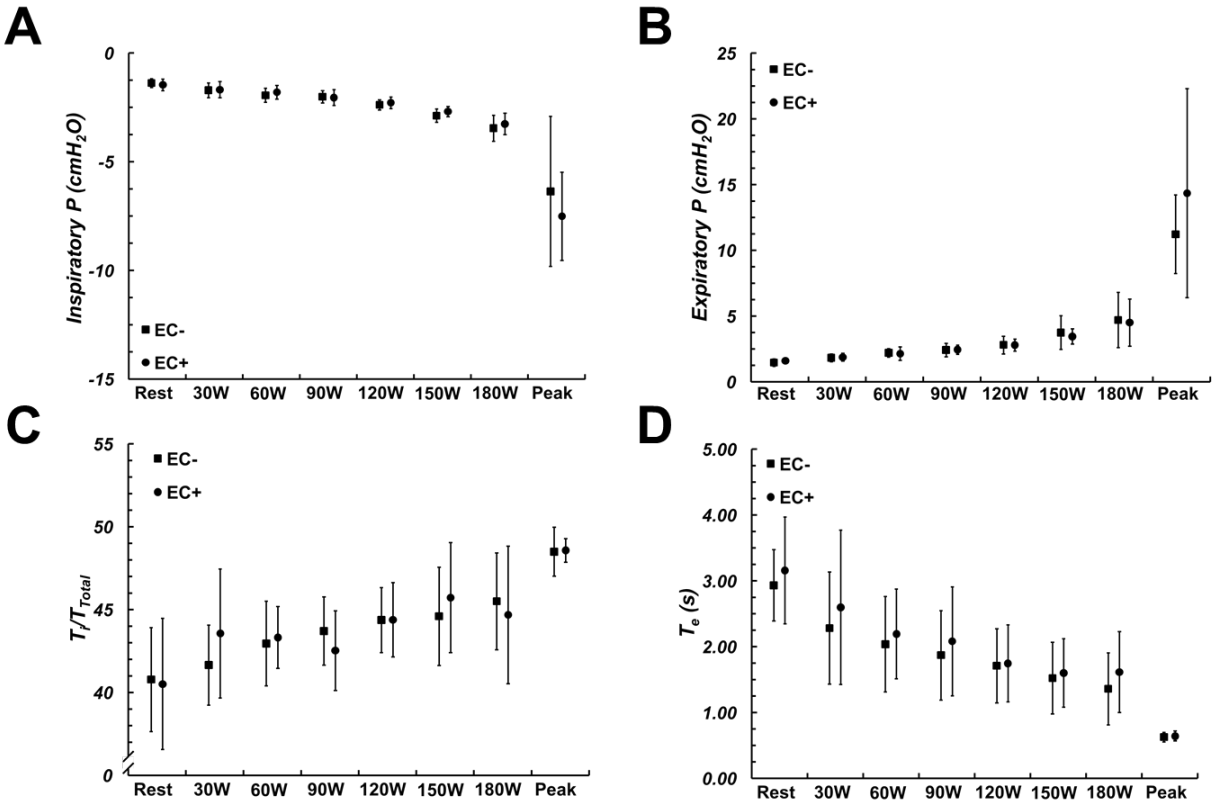

A
Inspiratory P (cmH2O)
0
-5
-10
-15
EC-
EC+
Rest
30W
60W
90W
120W
150W
180W
Peak
B
Expiratory P (cmH2O)
25
20
15
10
5
0
EC-
EC+
Rest
30W
60W
90W
120W
150W
180W
Peak
C
TI/TTotal
55
50
45
40
0
EC-
EC+
Rest
30W
60W
90W
120W
150W
180W
Peak
D
Te (s)
5.00
4.00
3.00
2.00
1.00
0.00
EC-
EC+
Rest
30W
60W
90W
120W
150W
180W
Peak

Figure 5.

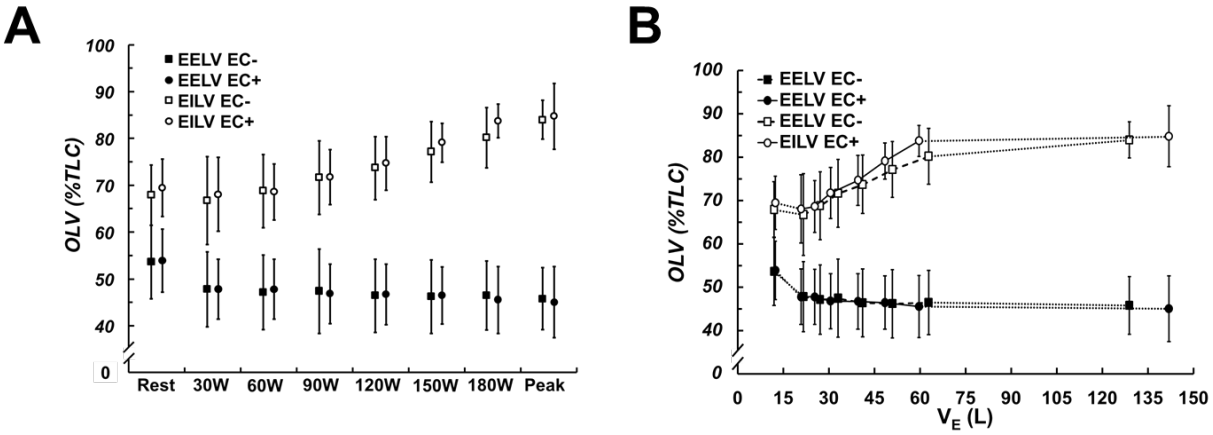
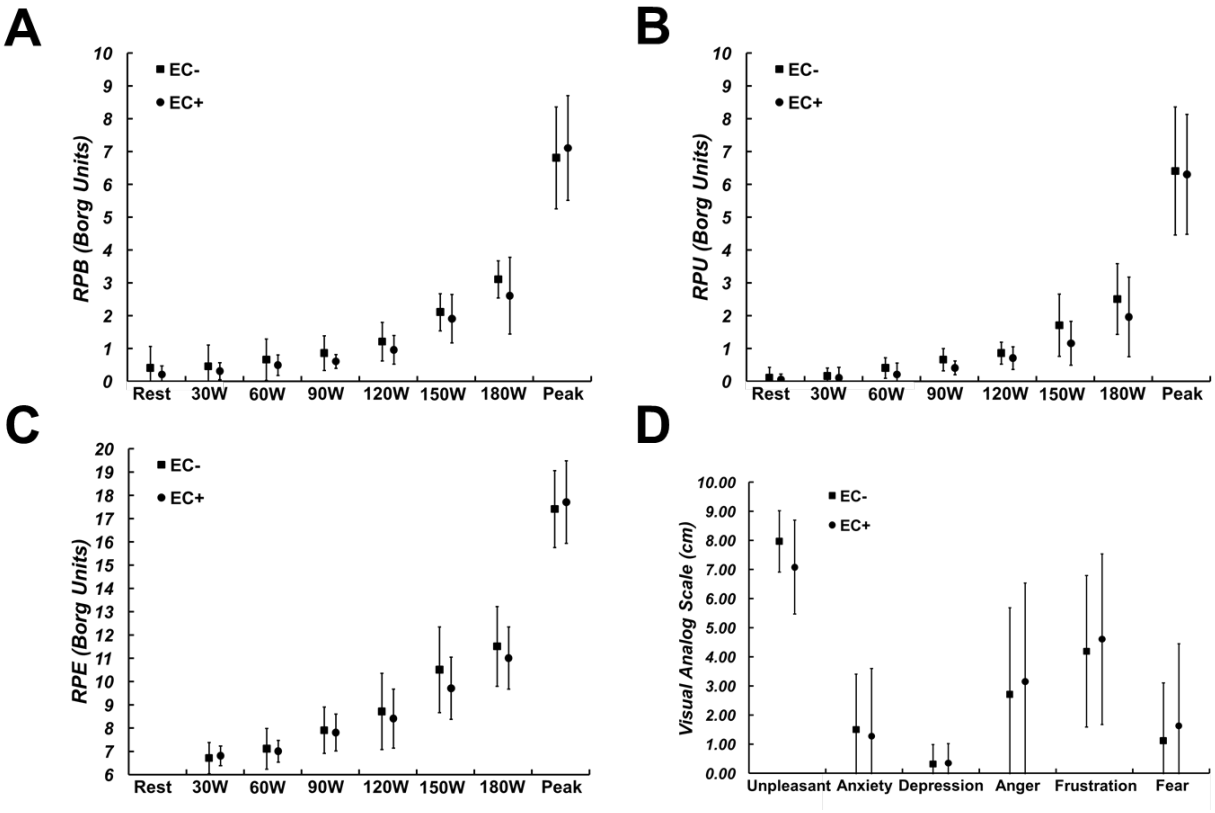

A
EELV EC-
EELV EC+
EILV EC-
EILV EC+
OLV (%TLC)
100
90
80
70
60
50
40
0
Rest
30W
60W
90W
120W
150W
180W
Peak
B
EELV EC-
EELV EC+
EELV EC-
EILV EC+
OLV (%TLC)
0
15
30
45
60
75
90
105
120
135
150
V_E (L)

Figure 6.


A
EC-
EC+
RPB (Borg Units)
0 1 2 3 4 5 6 7 8 9 10
Rest 30W 60W 90W 120W 150W 180W Peak
B
EC-
EC+
RPU (Borg Units)
0 1 2 3 4 5 6 7 8 9 10
Rest 30W 60W 90W 120W 150W 180W Peak
C
EC-
EC+
RPE (Borg Units)
6 7 8 9 10 11 12 13 14 15 16 17 18 19 20
Rest 30W 60W 90W 120W 150W 180W Peak
D
EC-
EC+
Visual Analog Scale (cm)
0.00 1.00 2.00 3.00 4.00 5.00 6.00 7.00 8.00 9.00 10.00
Unpleasant Anxiety Depression Anger Frustration Fear

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Appendices

Appendix A.a.: Formaldehyde study subject charactersitics

Study	ID Code	Elon or AppState	Date Studied	Progress Acute	Progress Chronic	Sex	DOB	Age - Study
FA	AlSh	Elon	2/4/2020	Complete	In Progress	F	7/16/1997	22.6
FA	AmTo	Elon	2/4/2020	Complete	In Progress	F	7/3/1996	23.6
FA	BrBu	Elon	2/6/2020	Complete	In Progress	F	12/26/1996	23.1
FA	CaRo	Elon	2/4/2020	Complete	In Progress	F	1/11/1994	26.1
FA	ErNo	Elon	2/6/2020	Complete	In Progress	F	5/21/1996	23.7
FA	GeAz	Elon	2/6/2020	Complete	In Progress	F	8/31/1997	22.4
FA	JoCh	Elon	2/4/2020	Complete	In Progress	F	8/1/1996	23.5
FA	KaHi	Elon	2/4/2020	Complete	In Progress	F	1/7/1996	24.1
FA	MaCo	Elon	2/4/2020	Complete	In Progress	F	8/13/1996	23.5
FA	MaJu	ASU	2/7/2020	Complete	In Progress	F	5/22/1998	21.7

Height (cm)	Weight (kg)	BMI	Ethnicity	Currently enrolled in other research	Overnight fast?	Blood draw	Fainting Hx	4hrs lying down	4hrs in chair
167.4	61.2	21.83939	Caucasian	No	Yes	Yes	No	Yes	No
155	61.2	25.47347	Caucasian	No	Yes	Yes	No	Yes	Yes
168.9	68.6	24.04722	Caucasian	No	Yes	Yes	No	Yes	Yes
157.48	48.08	19.38714	Caucasian	no	Yes	Yes	Yes	Yes	Yes
160	56.8	22.1875	Caucasian	No	Yes	Yes	No	Yes	Yes
162.56	52.27273	19.78098	Caucasian	No	Yes	Yes	No	Yes	Yes
165.1	58.97	21.63401	Caucasian	no	Yes	no	no	Yes	Yes
167.64	64	22.77323	Caucasian	No	Yes	Yes	No	Yes	Yes
160.02	63.63636	24.85174	Caucasian	No	Yes	Yes	No	Yes	Yes
162.56	70.45455	26.66132	Hispanic	No	Yes	Yes	No	Yes	Yes

Orthopeic issues?	Claustrophobic?	Physical activity?	Physically active (days/wk)	Activity duration (min)		Intensity	Type of activity	Exertional Sx	Health Hx
No	No	Yes	3	15-30	22.5	Vigorous	Run	.	.
No	No	Yes	4	45-60	52.5	Vigorous	ke, Run, Sw	.	.
No	No	Yes	4	60+	60	Vigorous	t Lifting an	No	opecia Area
No	No	Yes	4	15-30	22.5	Mild	Run, walk	.	.
No	No	Yes	3	45-60	52.5	Moderate	Bike, Weigl	No	Anemia
No	No	Yes	3	45-60	52.5	Vigorous	Weight lift,	iness, Dysp	No
No	No	Yes	4	45-60	52.5	Moderate	ke, WeightL	.	.
No	No	Yes	1	45-60	52.5	Moderate	Weight lift,	No	sthma, low
No	No	Yes	3	15-45	30	Mild	ke, Walk/Hi	No	TMJ
No	Yes	Yes	5	30-45	37.5	Moderate	alk/Hike, W	No	-

Current Sx	Allergies?	Allergy medication?	Ever had a stress test?	Surgery past 6 months?	Medication	Over-the-counter	Able to forego any Rx?	Willing to forego any Rx?	Pregnant?
.	.	.	.	.	No	No	Yes	Yes	No
.	no	No	No	No	No	No	.	.	No
No	No	No	No	No	No	Yes	Yes	Yes	No
.	Penicillin	.	no	no	.	no	no	no	no
No	No	No	No	No	pram, mina	Vitamin D	Yes	Yes	No
Glasses	No	No	No	No	No	dvil, Tylon	Yes	Yes	No
.	None	no	no	no	no	no	yes	yes	no
No	No	No	No	No	No	No	Yes	Yes	No
No	No	No	No	No	cline, Tri-P	No	Yes	No	No
No	No	No	No	No	-	-	-	-	No

Trying to get pregnant?	Contraceptives?		Contraceptive	Menopausal Status	Hormone replacement therapy?	Family Health Hx	Date Signed	Input by
No	Yes	1	Tri-Sprinte	Pre	No	P, High Cho	########	MAA
No	Yes	1		Pre	.	.	########	KCK
No	Yes	1	Tri-Sprinte	Pre	No	.	########	MAA
no	yes	1	rthcontrol p	pre	.	.	########	KCK
No	Yes	1	minastrin F	Pre	No	BP, Osteop	########	MAA
No	No	0		Pre	No	.	########	MAA
no	yes	1		pre	.	.	########	KCK
No	No	0		Pre	No	.	########	MAA
No	Yes	1	Tri-previfen	Pre	No	Arthritis	########	MAA
No	Yes	1	IUD-Mirena	Pre	No	t Attack, Obesity, High		SR

Appendix A.b.: Fomaldehyde exposure

	Date	Sensor	PA/PT	AM/PM	Highest FA Value
AdOg	02.04.2020		PA	AM	
AlSh	02.04.2020		PT	PM	
AmTo	02.04.2020		PA	AM	
BeLe	02.04.2020				
BrLe	02.04.2020		PA	AM	
CaGo	02.04.2020		PA	AM	
CaRo	02.04.2020		PA	AM	
GeAz	02.04.2020				
JoCh	02.04.2020		PA	AM	
KaHi	02.04.202(	FM-801-05	PT	PM	321
KaKa	02.04.2020		PT	PM	
KyGe	02.04.2020		PT	PM	
LaSy	02.04.202(	FM-801-05	PA	AM	
MaAc	02.04.202(	FM-801-05	PA	AM	
MaCo	02.04.2020		PT	PM	
StSc	02.04.2020		PA	AM	256

Time	**Baseline**	**Reading 1**	**Reading 2**	**Reading 3**	**Reading 4**
FM-801-01	7:45a	10:46a	11:16a	11:45a	12:16p
FM-801-02	9:04	10:46a	11:16a	11:45a	12:16p
FM-801-03	8:24a	10:45a	11:15a	11:45a	12:15p
FM-801-04	9:06a	10:45a	11:15a	11:45a	12:15p
FM-801-05	7:44a	10:45a	11:15a	11:45a	12:15p

[FA], ppb	**Baseline**	**Reading 1**	**Reading 2**	**Reading 3**	**Reading 4**
FM-801-01		117	**675**	545	254
FM-801-02		58	74	**99**	48
FM-801-03		138	**254**	220	86
FM-801-04		19	62	96	**103**
FM-801-05		180	**449**	356	165

2nd readings		**3:15p**	**3:45p**	**4:15p**	**4:45p**	**5:15p**
[FA], ppb	**Baseline**	**Reading 1**	**Reading 2**	**Reading 3**	**Reading 4**	**Reading 5**
FM-801-01		69	**320**	316	232	155
FM-801-02		27	196	231	**271**	139
FM-801-03		69	230	310	**344**	184
FM-801-04		13	193	**473**	389	161
FM-801-05		33	238	248	**321**	161

Appendix A.c.: Vascular function and biomarkers, formaldehyde

Pre-FA Exp	FA Levels			Biomarkers			
Subject	**[FA] Low**	**[FA] High**	**[FA] Avg**	**CRP, pg/mol**	**Protein Carb**	**Xanthine Oxi**	**TBARS ~MDA, µM**
LiEr							
OlMc							
MaJu							
Mean							
SE							
Subject	**[FA] Low**	**[FA] High**	**[FA] Avg**				
AlSh	196	271	232	258.847	0.0042079	3.952	4.209
AmTo	138	254	204	2477.5485	0.004895	73.342	6.315
BrBu	154	232	188	3079.348	0.00391875	0	2.945
CaRo	58	99	77	2592.338	0.00237875	0	5.6485
ErNo	225	356	278	2469.43	0.004345	35.239	6.526
GeAz	113	125	118	560.601	0.00482625	11.854	5.192
JoCh				97.999	0.0092125	0.2015	4.8405
KaHi	238	321	269				
MaCo	230	344	294	2878.424	0.0157575	0.989	4.4195
MaJu	20	219	113	745.297	0.016115	11.125	3.226
Mean	**152.44**	**246.78**	**197.00**	**1684.43**	**0.0073**	**15.19**	**4.81**
SD	78.04	90.27	79.22	1231.61	0.0052	24.56	1.25
Post-FA Exp							
Subject							
LiEr							
OlMc							
MaJu							
Mean							
SE							
AlSh				223.4925	0.0032725	0	7.79
AmTo				2783.5765	0.00345125	58.902	7.86
BrBu				3109.653	0.00862125	12.856	5.543
CaGo				2632.392	0.0033	2.6245	10.6335
ErNo				2324.11	0.0027225	104.866201	5.964
GeAz				455.5025	0.00284625	4.62894265	4.911
JoCh				68.877	0.0035475	0	6.315
KaHi							
MaCo				3150	0.02223375	0	4.1385
MaJu				3150	0.0037675	1.4965	3.6825
Mean				**1988.62**	**0.00597**	**20.60**	**6.32**
SD				1335.33	0.00636	36.82	2.17
				0.288	0.493	0.540	0.047
							0.31199289
Cohen's d				**0.24**	**-0.23**	**0.17**	**0.85**
Δ-FA Exp							
Subject							
LiEr							
OlMc							
MaJu							
Mean							
SE							
AlSh				-35.3545	-0.0009354	-3.952	3.581
AmTo				306.028	-0.0014438	-14.44	1.545
BrBu				30.305	0.0047025	12.856	2.598
CaGo				40.054	0.00092125	2.6245	4.985
ErNo				-145.32	-0.0016225	69.6272013	-0.562
GeAz				-105.0985	-0.00198	-7.2250573	-0.281
JoCh				-29.122	-0.005665	-0.2015	1.4745
KaHi							
MaCo				271.576	0.00647625	-0.989	-0.281
MaJu				2404.703	-0.0123475	-9.6285	0.4565
Mean				**304.20**	**-0.0013**	**5.41**	**1.50**
SE				802.65	0.0055	25.32	1.92

PWA					sPLM BF		
SBP	DBP	PP	MAP	HR	BL	Max	deltaPeak
106	77	29	88	61	791.035126	**1246.5549**	**455.519778**
108	75	33	90	68	421.793493	**899.471298**	**477.677805**
111	80	31	94	72	470.080912	**840.70869**	**370.627779**
109	79	30	92	66	472.795581	**903.778619**	**430.983038**
118	76	42	91	57	295.845682	**960.884679**	**665.038997**
97	72	25	83	59	552.264857	**1060.61515**	**508.350293**
97	73	24	85	73	725	**1141.72678**	**416.726784**
112	81	31	98	93	775.062325	**1229.78132**	**454.718999**
97	70	27	83	85	201.359823	**424.378359**	**223.018536**
106.11	**75.89**	**30.22**	**89.33**	**70.44**	**522.80**	**967.54**	**444.74**
7.59	3.76	5.31	5.10	12.02	208.46	251.37	117.00
111	72	39	86	59	480.06	**1237.22501**	**757.165014**
101	74	27	87	91	322.487161	**608.278996**	**285.791835**
110	82	28	95	72	423.71438	**759.478682**	**335.764302**
107	76	31	91	72	548.150093	**946.326493**	**398.1764**
107	75	32	87	70	326.532497	**533.425144**	**206.892647**
107	78	29	91	60	462.954224	**979.127329**	**516.173106**
112	81	31	97	79	905	**1320.95035**	**415.950353**
110	80	30	95	72	583.573291	**1068.58537**	**485.012082**
104	73	31	89	90	175.356552	**413.747582**	**238.39103**
107.67	**76.78**	**30.89**	**90.89**	**73.89**	**469.76**	**874.13**	**404.37**
3.54	3.63	3.44	4.01	11.29	206.06	315.71	169.17
0.591	0.546	0.761	0.439	0.411	0.304	0.160	0.565
0.26	**0.24**	**0.15**	**0.34**	**0.30**	**-0.26**	**-0.33**	**-0.28**
5	-5	10	-2	-2	-310.97513	-9.3298899	301.645236
-7	-1	-6	-3	23	-99.306332	-291.1923	-191.88597
-1	2	-3	1	0	-46.366532	-81.230008	-34.863477
-2	-3	1	-1	6	75.354512	42.5478734	-32.806639
-11	-1	-10	-4	13	30.6868152	-427.45954	-458.14635
10	6	4	8	1	-89.310633	-81.48782	7.82281316
15	8	7	12	6	180	179.223569	-0.7764313
-2	-1	-1	-3	-21	-191.48903	-161.19595	30.2930823
7	3	4	6	5	-26.003271	-10.630777	15.3724937
1.56	**0.89**	**0.67**	**1.56**	**3.44**	**-53.05**	**-93.42**	**-40.37**
8.34	4.23	6.36	5.73	11.91	144.80	181.08	202.04

		sPLM VC					cPLM BF		
AUC 60	AUC 30	BL	Max	deltaPeak	AUC 60	AUC 30	BL	Max	deltaPeak
80.5913286	40.9612032	8.98903552	**14.1653966**	**5.17636112**	0.91581055	0.46546822	531.273403	**1353.7418**	**822.468402**
324.491219	133.475538	4.68659437	**9.99412553**	**5.30753116**	3.60545799	1.48306154	439.048786	**1511.19988**	**1072.15109**
118.492275	106.712332	5.00086076	**8.94370947**	**3.94284871**	1.26055611	1.13523757	364.01889	**1134.55398**	**770.535086**
194.941279	79.7610532	5.1390824	**9.82368064**	**4.68459824**	2.11892694	0.86696797	769.278634	**1911.8479**	**1142.56927**
216.066855	152.273851	3.25105145	**10.5591723**	**7.30812085**	2.37436104	1.67333902	439.376129	**1105.55206**	**666.175931**
157.634989	137.036967	6.20522311	**11.9170242**	**5.71180104**	1.77117965	1.5397412	408.026215	**1118.19087**	**710.164651**
82.176151	92.5022003	8.52941176	**13.4320798**	**4.90266804**	0.96677825	1.08826118	643.579811	**1147.80666**	**504.226847**
16.2110522	69.2616021	7.90879924	**12.548789**	**4.63998979**	0.1654189	0.70675104	755.15	**1183.60424**	**428.454236**
56.8769778	47.8745827	2.42602197	**5.11299228**	**2.68697031**	4.04230393	21.9049972	265.87	**743.448536**	**477.578536**
138.61	**95.54**	**5.79**	**10.72**	**4.93**	**1.91**	**3.43**	**512.85**	**1245.55**	**732.70**
95.33	39.85	2.30	2.74	1.26	1.28	6.94	175.67	324.01	251.87
184.92979	157.886711	5.58209302	**14.3863374**	**8.80424435**	2.15034639	1.83589199	391.411943	**1002.81292**	**611.400978**
119.069138	71.2285063	3.50529523	**6.61172822**	**3.10643299**	1.29422977	0.77422289	484.32	**1171.87604**	**687.556036**
123.123922	102.219923	4.46015137	**7.99451244**	**3.53436107**	1.29604128	1.07599919	392.072979	**1241.20974**	**849.136761**
101.701918	100.791197	6.0236274	**10.3991922**	**4.37556483**	1.11760349	1.10759557	929.77427	**1987.05921**	**1057.28494**
47.151877	51.964709	3.7532471	**6.13132349**	**2.3780764**	0.5419756	0.59729551	308.136574	**1074.39465**	**766.258076**
260.106008	167.943361	5.32131292	**11.2543371**	**5.9330242**	2.98972423	1.93038346	610.807617	**1160.24299**	**549.435377**
78.1574665	112.061288	9.32989691	**13.6180449**	**4.28814796**	0.80574708	1.15527101	802.776708	**1179.85729**	**377.080586**
157.749785	100.764913	5.30521174	**9.71441248**	**4.40920074**	1.43408895	0.91604466	641.373403	**1069.83321**	**428.459811**
90.8076473	36.4459985	1.97029834	**4.64884924**	**2.6785509**	1.02031064	0.4095056	365.99	**913.999926**	**548.009926**
129.20	**100.15**	**5.03**	**9.42**	**4.39**	**1.41**	**1.09**	**547.41**	**1200.14**	**652.74**
64.00	43.72	2.05	3.36	1.98	0.74	0.51	214.15	311.72	213.88
0.824	0.828	0.159	0.061	0.492	0.376	0.359	0.462	0.489	0.173
-0.12	**0.11**	**-0.35**	**-0.43**	**-0.33**	**-0.49**	**-0.48**	**0.18**	**-0.14**	**-0.34**
104.338461	116.925508	-3.4069425	0.22094074	3.62788323	1.23453584	1.37042377	-139.86146	-350.92888	-211.06742
-205.42208	-62.247032	-1.1812991	-3.3823973	-2.2010982	-2.3112282	-0.7088386	45.2712142	-339.32384	-384.59505
4.63164691	-4.4924085	-0.5407094	-0.949197	-0.4084876	0.03548517	-0.0592384	28.054089	106.655764	78.6016753
-93.239361	21.0301436	0.88454499	0.57551158	-0.3090334	-1.0013235	0.2406276	160.495637	75.2113112	-85.284325
-168.91498	-100.30914	0.50219564	-4.4278488	-4.9300444	-1.8323854	-1.0760435	-131.23955	-31.15741	100.082145
102.471019	30.9063944	-0.8839102	-0.662687	0.22122316	1.21854458	0.39064226	202.781402	42.0521276	-160.72927
-4.0186845	19.5590879	0.80048514	0.18596506	-0.6145201	-0.1610312	0.06700983	159.196898	32.0506365	-127.14626
141.538733	31.5033105	-2.6035875	-2.8343765	-0.230789	1.26867005	0.20929362	-113.7766	-113.77102	0.00557436
33.9306695	-11.428584	-0.4557236	-0.464143	-0.0084194	-3.0219933	-21.495492	100.12	170.55139	70.4313899
-9.41	**4.61**	**-0.76**	**-1.30**	**-0.54**	**-0.51**	**-2.34**	**34.56**	**-45.41**	**-79.97**
123.10	61.58	1.48	1.79	2.25	1.63	7.22	134.19	187.94	160.21

AUC 60	AUC 30	cPLM VC BL	Max	deltaPeak	AUC 60	AUC 30	baseline diar	peak diamet
							3.29	3.4963
							3.004	3.2667
							3.7691	3.9767
							3.35	**3.58**
							0.22	0.21
436.769629	219.408266	6.03719777	**15.3834296**	**9.34623184**	4.96329124	2.49327575	4.10	4.63
667.099446	379.037537	4.87831984	**16.7911097**	**11.9127899**	7.41221606	4.21152819	3.77	3.73
567.923236	262.79827	3.87254138	**12.0697231**	**8.19718176**	6.04173656	2.79572628	3.18	3.48
621.164801	316.66543	8.36172428	**20.7809554**	**12.4192312**	6.75179131	3.44201554	2.99	3.29
125.157499	79.0256047	4.82830911	**12.1489237**	**7.32061463**	1.37535714	0.86841324	3.80	3.96
453.070108	226.536713	4.58456421	**12.5639423**	**7.97937811**	5.09067538	2.54535632	3.74	4.23
							2.98	3.50
386.643511	209.536215	7.57152718	**13.5036077**	**5.93208056**	4.54874718	2.46513194	3.04	3.28
217.476033	113.820202	7.70561224	**12.0775942**	**4.371982**	2.21914319	1.16143063	3.086	3.24
42.8805944	55.0696757	3.20325301	**8.95721128**	**5.75395826**	0.51663367	0.66349007	2.91	3.88
390.91	**206.88**	**5.67**	**13.81**	**8.14**	**4.32**	**2.29**	**3.36**	**3.72**
220.35	108.06	1.84	3.43	2.73	2.43	1.19	0.44	0.46

							baseline diar	peak diamet
							3.3086	3.6793
							2.837	3.175
							3.892	4.1012
							3.35	**3.65**
							0.31	0.27
360.58016	170.565423	4.55130166	**11.6606154**	**7.1093137**	4.19279255	1.98331888	3.76	3.98
183.480002	160.624198	5.26434783	**12.737783**	**7.47343518**	1.99434785	1.74591519	3.59	3.79
559.779218	304.95088	4.12708399	**13.0653657**	**8.93828169**	5.89241282	3.21000926	3.17	3.40
659.604316	403.866219	10.2172997	**21.8358155**	**11.6185158**	7.24839908	4.43809032	2.92	3.31
173.31912	113.670664	3.5417997	**12.3493638**	**8.80756409**	1.9921738	1.30655936	3.30	3.53
353.675133	184.833929	7.0207772	**13.3361264**	**6.31534917**	4.06523141	2.12452792	2.74	3.21
							3.27	3.39
256.567789	150.322818	8.27604854	**12.1634773**	**3.88742873**	2.64502875	1.54971978	2.81	3.25
159.362114	114.957983	5.8306673	**9.72575649**	**3.89508919**	1.44874649	1.04507257	3.101	3.30
73.6264729	123.807605	4.11224719	**10.2696621**	**6.1574149**	0.82726374	1.39109669	3.50	3.58
308.89	**191.96**	**5.88**	**13.02**	**7.13**	**3.37**	**2.09**	**3.21**	**3.47**
195.31	98.49	2.23	3.52	2.47	2.15	1.08	0.34	0.25
0.171	0.642	0.683	0.298	0.136	0.162	0.574	0.318	0.058
-0.39	**-0.14**	**0.10**	**-0.23**	**-0.39**	**-0.42**	**-0.18**	**-0.37**	**-0.67**
						0.70761479		

							baseline diar	peak diamet
							0.02	0.18
							-0.17	-0.09
							0.12	0.12
-76.18947	-48.842843	-1.4858961	-3.7228142	-2.2369181	-0.7704987	-0.5099569	-0.35	-0.65
-483.61944	-218.41334	0.38602798	-4.0533267	-4.4393547	-5.4178682	-2.465613	-0.18	0.06
-8.1440188	42.1526098	0.25454261	0.99564254	0.74109993	-0.1493237	0.41428298	-0.01	-0.08
38.4395155	87.2007891	1.85557539	1.05486007	-0.8007153	0.49660777	0.99607478	-0.08	0.02
48.1616208	34.6450595	-1.2865094	0.20044006	1.48694946	0.61681666	0.43814612	-0.49	-0.43
-99.394976	-41.702784	2.43621299	0.77218405	-1.6640289	-1.025444	-0.4208284	-1.01	-1.02
							0.29	-0.11
-130.07572	-59.213396	0.70452135	-1.3401305	-2.0446518	-1.9037184	-0.9154122	-0.23	-0.02
-58.113919	1.13778132	-1.8749449	-2.3518378	-0.4768928	-0.7703967	-0.1163581	0.02	0.06
30.7458785	68.7379294	0.90899418	1.31245081	0.40345663	0.31063007	0.72760662	0.59	-0.30
-82.02	**-14.92**	**0.21**	**-0.79**	**-1.00**	**-0.96**	**-0.21**	**-0.14**	**-0.25**
163.60	92.63	1.50	2.13	1.82	1.86	1.05	0.43	0.36
								0.00884697

λ diam	time of peak	time of peak	Sum Shear Vali	FMD(%) at pe	FMD/Shear	AUC
0.2063	80-90	80	98931.42282	6.27051672	0.06338246	398.089349
0.2627	40-50	40	67669.98843	8.74500666	0.12923021	287.662141
0.2076	0-4	0	3103.67893	5.50794619	1.77465077	579.105555
0.23		**40.00**	**56568.36**	**6.84**	**0.66**	**421.62**
0.02		23.09	28214.50	0.98	0.56	84.95
0.5322	60-70	60	56964.65	12.98	0.23	646.44
-0.0425	30-40	30	34536.46	2.26	0.07	249.63
0.2974	100-110	100	86804.31	9.36	0.11	369.68
0.294	60-70	60	110195.91	9.83	0.09	368.73
0.169	40-50	40	73258.52	4.45	0.06	641.61
0.487	40-50	40	73080.03	13.01	0.18	793.76
0.5176	80-90	80	147443.33	12.01	0.08	609.60
0.239	50-60	60	83248.65	15.00	0.25	294.21
0.154	50-60	50	60831.73	4.99	0.08	276.93
0.9705	40-50	40	91435.21	10.22	0.11	560.84
0.36		**56.00**	**81779.88**	**9.41**	**0.13**	**481.14**
0.28		21.19	31045.93	4.21	0.07	191.18

λ diam	time of peak	time of peak	Sum Shear Vali	FMD(%) at pe	FMD/Shear	AUC		
0.3707	80-90	80	133811.3773	10.8222892	0.0808772	629.537948		
0.338	40-50	40	121409.361	9.9871826	0.0822604	505.932944		
0.2092	80-90	80	92363.29677	5.37512847	0.0581955	729.84667		
0.31		**66.67**	**115861.35**	**8.73**	**0.07**	**621.77**		
0.05		13.33	12282.39	1.69	0.01	64.75		
0.229	50-60	50	94241.66	6.10	0.06	825.51	3.892	
0.2045	90-100	90	107110.08	5.69	0.05	551.13	4.1012	
0.2285	30-40	30	75718.38	7.21	0.10	465.09	80-90	
0.395	80-90	80	173055.94	8.51	0.08	556.47	92363.2968	
0.2262	100-110	100	89700.31	6.63	0.07	283.17	5.37512847	
0.4731	40-50	40	95031.84	8.14	0.09	350.24	0.0581955	
0.114	80-90	80	124152.15	7.84	0.06	454.86		
0.446	70-80	70	136323.82	11.43	0.12	472.22		Magnitude change maintair
0.1961	70-80	70	82143.58	3.47	0.04	341.70		
0.0835	30-40	30	89263.65	2.39	0.03	684.66		
0.26		**64.00**	**106674.14**	**6.74**	**0.07**	**498.51**		
0.13		25.03	29875.06	2.57	0.03	164.78		
0.371		0.515	0.035	0.043	0.016	0.831		
-0.47	**#DIV/0!**	**0.34**	**0.82**	**-0.77**	**-1.07**	**0.10**		

λ diam	time of peak	time of peak	Sum Shear Vali	FMD(%) at pe	FMD/Shear	AUC	FA Exposure
0.16		0.00	34879.95	4.55	0.02	231.45	29
0.08		0.00	53739.37	1.24	-0.05	218.27	29
0.00		80.00	89259.62	-0.13	-1.72	150.74	36
-0.30		-10.00	37277.01	-6.88	-0.16	179.07	271
0.25		60.00	72573.62	3.42	-0.01	301.50	
-0.07		-70.00	-11085.93	-2.15	-0.01	95.41	232
0.10		20.00	62860.04	-1.32	-0.01	187.74	219
0.06		60.00	16441.79	2.18	0.01	-358.43	
-0.01		0.00	21951.81	-4.87	-0.09	-443.52	125
-0.40		0.00	-23291.18	-4.17	-0.02	-154.74	
0.21		10.00	53075.17	-3.57	-0.13	178.01	321
0.04		20.00	21311.85	-1.52	-0.04	64.76	344
-0.89		-10.00	-2171.56	-7.84	-0.09	123.82	219
-0.10		**8.00**	**24894.26**	**-2.67**	**-0.06**	**17.36**	**247.29**
0.34		37.36	31805.18	3.60	0.06	250.48	73.19
0.02961792		0.07723059	0.138223815	0.3465506	0.14641547	4.5582E-05	

Appendix A.d.: Pulmonary function and formaldehyde

ID	GRP	STATUS	Code	AGE	HT (cm)	WT (kg)	SEX
AdOg	Elon	Complete	Pre	32	176.5	120.2	F
AlSh	Elon	Complete	Pre	22	168.0	60.2	F
AmTo	Elon	Complete	Pre	23	155.0	61.2	F
BrBu	Elon	Complete	Pre	23	170.0	68.6	F
BrLe	Elon	Complete	Pre	23	180.3	86.3	M
CaGo	Elon	Complete	Pre	23	165.1	66.7	F
CaRo	Elon	Complete	Pre	26	157.5	48.1	F
ErNo	Elon	Complete	Pre	23	160.0	60.0	F
GeAz	Elon	Complete	Pre	22	161.0	52.3	F
GrNe	Elon	Complete	Pre	32	185.0	81.8	M
JeTe	Elon	Complete	Pre	23	155.0	56.8	F
JoCh	Elon	Complete	Pre	23	165.1	59.0	F
KaHi	Elon	Complete	Pre	24	166.0	60.9	F
KaKa	Elon	Complete	Pre	23	173.0	75.5	F
KyGe	Elon	Complete	Pre	23	160.0	60.5	F
LaSy	Elon	Complete	Pre	24	167.6	58.1	F
LiKi	Elon	Complete	Pre	22	172.5	81.4	F
MaAc	Elon	Complete	Pre	23	162.6	60.0	F
MaCo	Elon	Complete	Pre	23	163.0	63.6	F
StSC	Elon	Complete	Pre	26	165.1	59.0	F
YuZh	Elon	Complete	Pre	24	170.5	66.4	F
LiEr	AppState	Complete	Pre	21	160.0	54.5	F
MaJu	AppState	Complete	Pre	21	162.6	70.5	F
OlMc	AppState	Complete	Pre	22	154.0	57.6	F
JeSu	AppState	DQ	Pre	22	154.9	63.6	F

SPIRO	FVC	FVC PP NHANES	FVC P Knudson	FVC PP Knudson	FEV1	FEV1 PP NHANES	FEV1 P Knudson	FEV1 PP Knudson
Spiro	4.13	107	4.080	101	3.54	110	3.406	104
Spiro	3.59	89	3.891	92	3.27	93	3.344	98
Spiro	3.75	109	3.318	113	2.82	94	2.922	96
Spiro	.	.	.	.	.	.	.	.
Spiro	5.74	100	5.451	105	4.93	104	4.702	105
Spiro	.	.	.	.	.	.	.	.
Spiro	.	.	.	.	.	.	.	.
Spiro	2.81	77	3.532	80	2.42	76	3.077	79
Spiro	3.41	92	3.592	95	3.07	95	3.128	98
Spiro	5.44	92	5.879	93	4.14	87	4.853	85
Spiro	3.31	97	3.318	100	3.02	100	2.922	103
Spiro	.	.	.	.	.	.	.	.
Spiro	3.64	90	3.771	97	2.79	80	3.242	86
Spiro	4.35	101	4.087	106	3.95	107	3.478	114
Spiro	3.62	115	3.533	103	2.86	103	3.077	93
Spiro	3.77	93	3.839	98	2.85	82	3.291	87
Spiro	4.88	113	4.083	120	4.31	116	3.483	124
Spiro	.	.	.	.	.	.	.	.
Spiro	.	.	.	.	.	.	.	.
Spiro	.	.	.	.	.	.	.	.
Spiro	3.96	94	3.963	100	3.41	95	3.381	101
Spiro	3.85	105	3.567	108	3.20	100	3.118	103
Spiro	3.52	91	3.676	96	3.12	92	3.196	98
Spiro	2.95	87	3.293	90	2.88	96	2.911	99
Spiro	.	.	.	.	.	.	.	.

FEV1/FVC	FEV1/FVC PP Knudson	25-75% (L/s)	25-75% PP	FEF25	FEF50	FEF75	PEF (L/min)	PEF PP
85.77	102.75	4.59	129	8.44	6.29	1.71	9.35	120
90.90	105.76	4.61	121	6.71	5.22	2.30	8.45	117
75.06	85.23	2.26	65	4.77	2.86	1.08	6.91	107
.	.	.	.	.	.	.	.	.
86.02	99.71	5.97	122	10.03	7.91	2.64	11.54	111
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.
85.88	98.58	2.99	83	5.02	3.94	1.18	6.29	93
90.04	103.40	3.71	101	6.24	4.26	1.97	6.78	100
76.09	92.16	3.32	71	7.15	3.72	2.63	11.52	106
91.12	103.47	4.44	127	6.75	5.30	2.19	7.14	111
.	.	.	.	.	.	.	.	.
76.65	89.15	2.36	63	4.31	2.78	1.16	5.66	78
90.85	106.74	5.18	132	8.10	5.79	3.12	8.54	113
78.87	90.53	2.55	75	4.56	3.14	1.29	6.49	96
75.70	88.29	2.45	65	4.17	2.87	1.20	5.89	81
88.31	103.51	5.08	129	6.59	5.26	3.48	7.43	99
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.
86.00	100.79	3.96	103	6.37	5.09	1.90	6.69	90
83.20	95.21	3.54	97	5.20	3.79	2.01	5.78	86
88.65	101.95	3.59	92	6.07	3.98	1.87	7.38	106
97.58	110.37	4.10	117	7.57	4.25	2.20	7.75	122
.	.	.	.	.	.	.	.	.

FET100%	FIVC	FIVC PP NHANES	PIF	FIF25	FIF50	FIF75	MVV	MVV PP	fb	VT (L)
6.46	3.36	87	6.26	6.13	6.01	5.03	135	110	79.3	1.67
4.06	3.01	74	5.07	4.82	5.01	4.69	129	106	99.7	1.31
6.93	3.10	90.47593	3.17	2.20	2.47	2.63	105	95	87.4	1.26
.	.	.	.	.	.	.	.	.	.	.
6.25	4.84	85	6.83	6.04	6.77	6.68	196	124	87.7	2.25
.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.
7.30	2.42	66	3.87	2.52	3.09	3.08	103	90	111	0.94
2.35	2.77	75	2.90	0.25	0.06	0.06	85	74	108.6	0.78
6.24	5.00	84	4.84	4.11	4.00	3.08	.	.	.	.
6.81	2.96	86	4.12	3.26	2.71	1.49	127	115	95.2	1.34
.	.	.	.	.	.	.	.	.	.	.
5.89	2.90	72	3.31	3.21	2.85	2.07	97	82	99.4	0.99
6.33	3.48	81	5.11	4.49	4.88	4.71	131	105	102.9	1.29
4.73	2.68	85	3.53	3.21	3.18	2.38	146	128	116.5	1.28
4.96	3.06	76	3.64	3.40	3.50	2.93	94	78	83.2	1.17
7.63	4.09	95	3.71	2.76	2.66	2.71	124	99	121.8	1.02
.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.	.
6.40	3.16	75	3.01	2.46	2.82	2.52	134	109	129.6	1.06
5.03	3.17	87	3.87	3.46	3.53	3.15	101	87	98.3	1.04
3.26	2.88	75	4.52	3.00	3.89	4.41	138	117	121.2	1.18
3.49	2.36	70	3.74	0.61	0.96	1.10	114	104	105.9	1.06
.	.	.	.	.	.	.	.	.	.	.

ID	GRP	STATUS	Code	AGE	HT (cm)	WT (kg)	SEX
AdOg	Elon	Complete	Post	32	176.5	120.2	F
AlSh	Elon	Complete	Post	22	168.0	60.2	F
AmTo	Elon	Complete	Post	23	155.0	61.2	F
BrBu	Elon	Complete	Post	23	170.0	68.6	F
BrLe	Elon	Complete	Post	23	180.3	86.3	M
CaGo	Elon	Complete	Post	23	165.1	66.7	F
CaRo	Elon	Complete	Post	26	157.5	48.1	F
ErNo	Elon	Complete	Post	23	160.0	60.0	F
GeAz	Elon	Complete	Post	22	161.0	52.3	F
GrNe	Elon	Complete	Post	32	185.0	81.8	M
JeTe	Elon	Complete	Post	23	155.0	56.8	F
JoCh	Elon	Complete	Post	23	165.1	59.0	F
KaHi	Elon	Complete	Post	24	166.0	60.9	F
KaKa	Elon	Complete	Post	23	173.0	75.5	F
KyGe	Elon	Complete	Post	23	160.0	60.5	F
LaSy	Elon	Complete	Post	24	167.6	58.1	F
LiKi	Elon	Complete	Post	22	172.5	81.4	F
MaAc	Elon	Complete	Post	23	162.6	60.0	F
MaCo	Elon	Complete	Post	23	163.0	63.6	F
StSC	Elon	Complete	Post	26	165.1	59.0	F
YuZh	Elon	Complete	Post	24	170.5	66.4	F
LiEr	AppState	Complete	Post	21	160.0	54.5	F
MaJu	AppState	Complete	Post	21	162.6	70.5	F
OlMc	AppState	Complete	Post	22	154.0	57.6	F
JeSu	AppState	DQ	Post	22	154.9	63.6	F

SPIRO	FVC	FVC PP NHANES	FVC P Knudson	FVC PP Knudson	FEV1	FEV1 PP NHANES	FEV1 P Knudson	FEV1 PP Knudson	FEV1/FVC
Spiro	4.22	109	4.080	104	3.59	111	3.406	105	84.97
Spiro	3.60	89	3.891	92	3.23	92	3.344	96	89.72
Spiro	3.58	104	3.318	108	2.72	90	2.922	93	75.94
Spiro	.	.	.	.	.	.	.	.	.
Spiro	5.78	101	5.451	106	4.96	105	4.702	105	85.82
Spiro	.	.	.	.	.	.	.	.	.
Spiro	.	.	.	.	.	.	.	.	.
Spiro	2.78	76	3.532	79	2.40	75	3.077	78	86.44
Spiro	3.34	90	3.592	93	2.89	89	3.128	92	86.46
Spiro	5.87	99	5.879	100	4.48	94	4.853	92	76.31
Spiro	3.29	96	3.318	99	2.93	97	2.922	100	89.12
Spiro	.	.	.	.	.	.	.	.	.
Spiro	3.62	89	3.771	96	2.86	82	3.242	88	78.87
Spiro	4.25	99	4.087	104	3.74	101	3.478	108	88.05
Spiro	3.53	112	3.533	100	3.21	116	3.077	104	91.05
Spiro	3.73	92	3.839	97	2.82	81	3.291	86	75.59
Spiro	4.75	111	4.083	116	4.19	113	3.483	120	88.15
Spiro	.	.	.	.	.	.	.	.	.
Spiro	.	.	.	.	.	.	.	.	.
Spiro	.	.	.	.	.	.	.	.	.
Spiro	3.94	94	3.963	99	3.41	95	3.381	101	86.58
Spiro	3.79	104	3.567	106	3.19	99	3.118	102	84.23
Spiro	3.56	92	3.676	97	3.10	92	3.196	97	87.03
Spiro	2.99	89	3.293	91	2.92	98	2.911	100	97.47
Spiro	.	.	.	.	.	.	.	.	.

FEV1/FVC PP Knudson	25-75% (L/s)	25-75% PP	FEF25	FEF50	FEF75	PEF (L/min)	PEF PP	FET100%
101.79	4.31	121	8.95	6.03	1.88	9.73	125	5.96
104.39	4.50	118	7.11	5.45	2.21	7.75	107	5.50
86.23	2.21	63	4.79	2.70	0.99	6.98	108	6.78
.	.	.	.	.	.	.	.	.
99.48	5.77	118	10.07	7.91	2.59	11.98	116	6.59
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.
99.22	2.83	78	5.03	3.38	1.25	5.65	84	7.04
99.28	2.88	79	4.26	3.15	1.57	5.44	80	2.98
92.43	3.72	80	6.63	4.63	1.91	11.03	101	5.81
101.20	4.11	117	6.10	5.05	1.87	7.02	109	6.92
.	.	.	.	.	.	.	.	.
91.72	2.55	68	4.56	3.14	1.29	6.49	89	4.73
103.45	5.18	133	8.42	6.59	2.16	8.71	115	3.42
104.52	5.19	153	8.15	6.37	2.41	9.87	146	4.75
88.16	2.36	63	4.05	2.65	1.35	5.88	81	6.40
103.34	4.03	102	5.24	4.65	2.59	6.91	92	6.79
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.
101.47	4.11	107	7.03	5.45	1.90	7.45	100	5.89
96.38	3.57	97	5.52	4.23	1.66	6.19	92	4.91
100.10	3.56	91	6.51	4.20	1.81	7.30	105	4.06
110.24	4.68	133	6.84	5.88	2.29	7.22	113	1.82
.	.	.	.	.	.	.	.	.

FIVC	FIVC PP NHANES	PIF	FIF25	FIF50	FIF75	MVV	MVV PP	fb	VT (L)
3.50	91	6.23	5.94	5.69	4.79	132	108	78.5	1.68
3.11	77	4.95	4.48	4.73	4.61	119	98	93.1	1.31
2.98	86.95893	2.28	1.79	2.01	2.19	114	104	101.6	1.17
.	.	.	.	.	.	.	.	.	.
4.88	85	7.53	6.77	7.24	6.19	230	146	118.6	2.00
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
2.34	64	4.19	3.32	3.47	3.36	101	89	119.3	0.86
2.77	75	2.69	0.56	1.17	1.29	94	81	89.8	1.05
5.04	85	4.35	1.20	2.35	2.13	.	.	.	.
2.63	77	3.37	2.62	2.37	1.69	121	110	106.3	1.14
.	.	.	.	.	.	.	.	.	.
2.68	66	3.53	3.21	3.18	2.38	91	77	111.9	0.83
3.38	78	5.04	4.52	4.59	3.85	125	100	84.1	1.49
3.01	95	3.99	3.76	3.15	2.25	148	130	124.8	1.21
3.11	77	4.07	4.00	3.92	2.99	93	78	83.7	1.21
3.96	92	3.24	2.24	2.82	3.03	131	104	126.6	1.06
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
.	.	.	.	.	.	.	.	.	.
3.25	77	3.30	2.52	2.48	2.18	141	115	109.6	1.30
3.13	85	4.13	4.01	3.39	2.84	107	92	97.9	1.10
2.98	77	5.26	2.88	3.43	3.69	142	120	143.8	0.99
2.40	71	4.17	3.71	3.75	3.39	105	96	101.1	1.04
.	.	.	.	.	.	.	.	.	.

Appendix A.e.: Electronic cigarette subject characteristics

LN	STUDY	ID	GRP	STATUS	REASON	RACE	AGE	DOB	SEX
2	ECIG	201	Naïve	Complete	.	white	20	4/19/1996	M
3	ECIG	202	User	Complete	.	white	20	2/4/1997	M
5	ECIG	204	User	Complete	.	hispanic	19	11/8/1997	M
6	ECIG	205	User	Complete	.	white	19	2/4/1998	M
9	ECIG	208	User	Complete	.	white	19	8/11/1997	M
12	ECIG	211	Naïve	Complete	.	white	19	2/6/1998	M
15	ECIG	214	Naïve	Complete	.	white	20	5/4/1997	M
16	ECIG	215	Naïve	Complete	.	white	23	4/6/1994	M
17	ECIG	216	User	Complete	.	white	25	10/18/1991	M
19	ECIG	218	Naïve	Complete	.	white	22	10/28/1995	M

CODE	HT (cm)	WT (kg)	WT:HT	BMI	HxDOE	snore-subjective	snore-objective	Hx Asth	Asthma Info	Smoke HX	Pks/day	Years Sm
Mean	198.1	106.6	0.538	27.2	N	N	N	N	N	0	0	0
Mean	166.1	78.9	0.475	28.6	N	Y	Y	Y	N	1	0	0
Mean	176.0	93.0	0.528	30.0	N	Y	Y	N	N	0	0	0
Mean	182.1	98.0	0.538	29.5	N	Y	Y	N	N	1	0.5	0.5
Mean	190.0	74.8	0.394	20.7	Y	N	Y	N	N	1	0.2	1
Mean	179.1	73.0	0.408	22.8	N	N	Y	N	N	0	0	0
Mean	182.9	76.2	0.417	22.8	N	N	N	N	N	0	0	0
Mean	184.9	91.6	0.496	26.8	N	N	N	N	N	0	0	0
Mean	172.0	63.0	0.367	21.3	N	Y	Y	N	N	1	0.3	6
Mean	193.0	83.9	0.435	22.5	N	Y	Y	N	N	0	0	0

Pks/yr	IPAQ Vigorus (days/wk)	IPAQ Vigorus (min/day)	IPAQ Moderate (days/wk)	IPAQ moderate (min/day)	IPAQ Walking(days/wk)	IPAQ walking (min/day)	IPAQ Sitting (min/day)	IPAQ (METmin/ wk)	Ex	Type Ex	ExFreq (per/wk)
0	0	0	1	60	4	30	600	636	Y	BASKETBALL	1
0	5	90	0	0	1	60	.	3798	Y	Run & Weight lifting	5
0	0	1	4	60	7	0	.	960	N	.	0
0.25	3	120	4	60	7	60	120	5226	Y	Cardio & Weights	3
0.2	4	90	2	90	7	120	255	6372	Y	Run & Bike	3
0	5	90	6	45	5	45	390	5422.5	Y	RUN	1
0	0	0	1	120	7	30	480	1173	Y	Run	2-4
0	3	60	4	60	5	10	660	2565	Y	RUN & BIKE	3
1.8	0	0	2	120	7	180	300	5118	N	.	0
0	.	.	.	.	.	.	.	.	Y	Sports & Swim	often

ExDuration (min)	Meds	Processing
60-120	N	MAA
90.0	ritalin - methylplenidate_ 1/day as needed	MAA
0.0	N	MAA
120.0	Lexapro 10mg as needed	MAA
30.0	Fluticasone Propionate Nasal Spray _ Allergy Relief	MAA
10.0	N	MAA
10.0	Flonase	MAA
60.0	N	MAA
0.0	Omega -3 600mg twice daily	MAA
F	N	MAA

Appendix A.f.: Electronic cigarette familiarization, pulmonary function

ID	GRP	STATUS	Code	AGE	HT (cm)	WT (kg)	SEX	SPIRO	FVC	FVC PP NHANES	FVC P Knudson
201	Naïve	Complete	.	20	198.1	106.6	M	Spiro	8.03	114	6.28
202	User	Complete	.	20	166.1	78.9	M	Spiro	5.12	106	4.39
204	User	Complete	.	19	176.0	93.0	M	Spiro	5.42	102	4.90
205	User	Complete	.	19	182.1	98.0	M	Spiro	6.04	106	5.26
208	User	Complete	.	19	190.0	74.8	M	Spiro	6.58	105	5.73
211	Naïve	Complete	.	19	179.1	73.0	M	Spiro	5.76	105	5.08
214	Naïve	Complete	.	20	182.9	76.2	M	Spiro	6.73	113	5.38
215	Naïve	Complete	.	23	184.9	91.6	M	Spiro	6.49	107	5.72
216	User	Complete	.	25	172.0	63.0	M	Spiro	5.80	112	4.99
218	Naïve	Complete	.	22	193.0	83.9	M	Spiro	6.68	101	6.13

FVC PP Knudson	FEV1	FEV1 PP NHANES	FEV1 P Knudson	FEV1 PP Knudson	FEV1/FVC	FEV1/FVC PP Knudson	25-75%	25-75% PP	PEF	PEF PP	FET100%	MVV
128	6.25	109	5.44	115	77.83	90	5.38	93	12.83	107	10.37	208
117	3.92	95	3.78	104	76.56	89	3.02	66	10.18	112	7.90	158
111	4.10	92	4.23	97	75.65	88	3.25	68	10.15	107	13.62	172
115	4.95	104	4.54	109	81.95	95	4.98	100	11.72	116	8.62	209
115	6.01	116	4.95	121	91.34	106	6.30	119	9.65	89	6.10	189
113	5.04	109	4.38	115	87.50	101	5.90	121	10.92	111	7.06	199
125	5.00	101	4.65	108	74.29	86	4.18	81	9.24	88	5.40	182
113	5.32	107	4.94	108	81.97	95	5.58	110	12.62	117	10.55	227
116	4.51	105	4.19	108	77.76	93	3.91	87	10.36	107	6.90	196
109	5.38	99	5.30	102	80.54	93	5.01	92	13.23	115	9.73	203

MVV PP	fb	LUNG VOL	TLC	TLC P G/B	TLC PP G/B	TLC P G/B Corrected	TLC PP G/B Corrected	TLC P ATS/ERS	TLC PP ATS/ERS	TLC P ATS/ERS Corrected	TLC PP ATS/ERS Corrected
90	85	LungVol	8.72	9.16	95	.	.	8.75	100	.	.
96	90	LungVol	5.74	6.15	93	.	.	6.19	93	.	.
93	80	LungVol	5.81	7.09	82	.	.	6.98	83	.	.
106	90	LungVol	7.26	7.67	95	.	.	7.47	97	.	.
89	90	LungVol	8.85	8.41	105	.	.	8.10	109	.	.
105	110	LungVol	6.94	7.38	94	.	.	7.23	96	.	.
92	95	LungVol	7.76	7.72	100	.	.	7.53	103	.	.
114	105	LungVol	7.52	7.87	96	.	.	7.69	98	.	.
114	80	LungVol	7.34	6.62	111	.	.	6.66	110	.	.
94	90	LungVol	9.99	8.65	116	.	.	8.34	120	.	.

Vtg	FRC PL	FRC %TLC	Pred FRC %TLC	PP FRC %TLC	FRC Pred G/B	FRC PP G/B	FRC P G/B Corrected	FRC PP G/B Corrected	FRC P ATS/ERS	FRC PP ATS/ERS	FRC P ATS/ERS Corrected
4.41	5.02	58	48	120	4.60	109	.	.	3.73	135	.
2.99	2.91	51	48	106	2.93	99	.	.	2.98	98	.
3.55	3.48	60	48	125	3.33	105	.	.	3.20	109	.
3.80	3.26	45	48	94	3.65	89	.	.	3.34	97	.
5.66	5.18	59	48	123	4.72	110	.	.	3.53	147	.
4.02	3.22	46	48	97	3.98	81	.	.	3.27	98	.
5.07	4.95	64	48	133	4.18	119	.	.	3.37	147	.
3.91	3.77	50	49	103	3.98	95	.	.	3.44	109	.
4.32	3.99	54	49	111	3.71	107	.	.	3.16	126	.
5.15	4.05	41	48	84	4.72	86	.	.	3.63	112	.

FRC PP ATS/ERS Corrected	ERV	RV	RV P G/B	RV PP G/B	RV P G/B Corrected	RV PP G/B Corrected	RV P ATS/ERS	RV PP ATS/ERS	RV P ATS/ERS Corrected	RV PP ATS/ERS Corrected	RV/TLC	VC
.	3.98	0.75	2.24	33	.	.	1.81	42	.	.	9	7.97
.	1.96	0.74	1.38	53	.	.	1.39	53	.	.	13	5.00
.	2.91	0.48	1.63	29	.	.	1.49	32	.	.	8	5.33
.	1.84	1.33	1.79	74	.	.	1.57	84	.	.	18	5.93
.	2.68	2.46	2.01	123	.	.	1.68	147	.	.	28	6.39
.	1.82	1.36	1.71	79	.	.	1.53	89	.	.	20	5.58
.	3.35	1.48	1.83	81	.	.	1.61	92	.	.	19	6.28
.	2.93	0.83	1.94	43	.	.	1.70	49	.	.	11	6.69
.	2.24	1.71	1.62	105	.	.	1.57	109	.	.	23	5.63
.	1.20	2.19	2.14	102	.	.	1.78	123	.	.	22	7.80

VC PP	IC	IC P G/B	IC PP G/B	IC P G/B Corrected	IC PP G/B Corrected	IC P ATS/ERS	IC PP ATS/ERS	IC P ATS/ERS Corrected	IC PP ATS/ERS Corrected	DLCO	DLCO PP Burrows	DLCO PP Miller
114	3.70	4.56	81	.	.	5.02	74	.	.	.	.	.
103	2.83	3.22	88	.	.	3.22	88	.	.	.	.	.
101	2.33	3.77	62	.	.	3.78	62	.	.	.	.	.
104	4.00	4.02	100	.	.	4.13	97	.	.	41.6	113	104
102	3.67	3.69	99	.	.	4.57	80	.	.	45.2	136	110
102	3.72	3.40	109	.	.	3.96	94	.	.	32.8	103	83
106	2.80	3.55	79	.	.	4.16	67	.	.	.	.	.
110	3.75	3.89	96	.	.	4.25	88	.	.	44.8	128	112
109	3.35	2.91	115	.	.	3.50	96	.	.	35.8	129	95
118	5.94	3.92	151	.	.	4.72	126	.	.	.	.	.

DLCO P ATS/ERS	DLCO PP ATS/ERS	DLco/VA	DLco/VA PP Burrows	DLco/VA PP Burrows STPD	DLco/VA PP Miller	DLco/VA PP ATS/ERS	VA	VA %TLC	VA %Pred TLC G/B	IVC	Raw	Raw PP
43	.	.	.	.	.	.	.	.	.	.	1.76	192
32	.	.	.	.	.	.	.	.	.	.	3.19	203
35	.	.	.	.	.	.	.	.	.	.	1.80	138
38	111	6.04	142	102	105	120	6.88	94.74	89.67	5.76	2.53	213
40	113	5.28	123	89	94	106	8.56	96.63	101.76	6.01	1.29	143
37	90	4.92	113	83	84	97	6.67	96.11	90.37	5.35	1.45	132
38	.	.	.	.	.	.	.	.	.	.	1.64	158
38	116	5.98	140	103	107	120	7.50	99.67	95.24	5.95	1.61	148
34	105	4.91	116	85	85	96	7.30	99.43	110.16	5.46	1.36	113
41	.	.	.	.	.	.	.	.	.	.	0.96	106

Gaw	Gaw PP	sRaw	sRaw PP	sGaw	sGaw PP	Pimax	Pemax
0.567	51	7.77	183	0.129	55	.	.
0.315	44	9.48	201	0.106	50	.	.
0.562	68	6.40	142	0.159	72	.	.
0.396	44	9.63	217	0.104	46	.	.
0.777	69	7.30	173	0.137	58	.	.
0.688	72	5.84	134	0.171	75	.	.
0.610	61	8.32	192	0.120	52	.	.
0.621	64	6.29	144	0.159	69	.	.
0.736	83	5.87	132	0.170	76	.	.
1.050	93	4.92	116	0.204	86	.	.

Appendix A.g.: Electronic cigarette placebo, pulmonary function

ID	GRP	STATUS	Code	AGE	HT (cm)	WT (kg)	SEX	SPIRO	FVC	FVC PP NHANES	FVC P Knudson
201	Naïve	Complete	.	20	198.1	107.0	M	Spiro	7.68	109	6.28
202	User	Complete	.	20	166.1	79.0	M	Spiro	4.87	100	4.39
204	User	Complete	.	19	176.0	90.0	M	Spiro	4.93	93	4.90
205	User	Complete	.	19	182.1	100.0	M	Spiro	6.05	106	5.26
208	User	Complete	.	19	190.0	77.6	M	Spiro	6.69	107	5.73
211	Naïve	Complete	.	19	179.1	74.8	M	Spiro	5.72	104	5.08
214	Naïve	Complete	.	20	182.9	75.6	M	Spiro	6.64	112	5.38
215	Naïve	Complete	.	23	184.9	90.6	M	Spiro	6.13	101	5.72
216	User	Complete	.	25	172.0	63.0	M	Spiro	5.48	106	4.99
218	Naïve	Complete	.	22	193.0	83.9	M	Spiro	6.47	98	6.13

FVC PP Knudson	FEV1	FEV1 PP NHANES	FEV1 P Knudson	FEV1 PP Knudson	FEV1/FVC	FEV1/FVC PP Knudson	25-75%	25-75% PP	PEF	PEF PP	FET100%	MVV
122	5.68	99	5.44	104	73.96	85	4.56	79	11.31	94	11.32	201
111	3.53	86	3.78	94	72.48	84	2.60	57	8.93	99	8.15	159
101	4.21	94	4.23	100	85.40	99	4.47	94	10.39	109	7.05	179
115	5.14	108	4.54	113	84.96	98	5.75	115	11.92	118	7.31	194
117	6.12	118	4.95	124	91.48	106	6.35	120	10.28	95	6.87	210
113	4.83	105	4.38	110	84.44	98	5.15	106	10.06	103	5.73	205
123	4.89	99	4.65	105	73.64	85	3.87	75	10.52	100	6.96	196
107	5.09	102	4.94	103	83.03	96	5.58	110	10.90	101	8.97	230
110	4.18	97	4.19	100	76.28	91	3.42	76	9.30	96	7.00	189
106	5.31	98	5.30	100	82.07	95	5.10	93	10.84	94	10.78	229

MVV PP	fb	LUNG VOL	TLC	TLC P G/B	TLC PP G/B	TLC P G/B Corrected	TLC PP G/B Corrected	TLC P ATS/ERS	TLC PP ATS/ERS	TLC P ATS/ERS Corrected	TLC PP ATS/ERS Corrected
87	85	LungVol	8.61	9.16	94	.	.	8.75	98	.	.
97	100	LungVol	5.40	6.15	88	.	.	6.19	87	.	.
97	85	LungVol	6.24	7.09	88	.	.	6.98	89	.	.
98	90	LungVol	7.37	7.67	96	.	.	7.47	99	.	.
99	90	LungVol	9.20	8.41	109	.	.	8.10	114	.	.
108	80	LungVol	6.45	7.38	87	.	.	7.23	89	.	.
99	85	LungVol	8.11	7.72	105	.	.	7.53	108	.	.
115	110	LungVol	7.65	7.87	97	.	.	7.69	99	.	.
110	90	LungVol	7.40	6.62	112	.	.	6.66	111	.	.
106	100	LungVol	9.23	8.65	107	.	.	8.34	111	.	.

Vtg	FRC PL	FRC %TLC	Pred FRC %TLC	PP FRC %TLC	FRC Pred G/B	FRC PP G/B	FRC P G/B Corrected	FRC PP G/B Corrected	FRC P ATS/ERS	FRC PP ATS/ERS	FRC P ATS/ERS Corrected
5.47	4.49	52	48	109	4.59	98	.	.	3.73	121	.
3.44	2.72	50	48	105	2.92	93	.	.	2.98	91	.
3.72	2.28	37	48	76	3.39	67	.	.	3.20	71	.
3.65	2.90	39	48	82	3.61	80	.	.	3.34	87	.
5.24	4.74	52	48	108	4.65	102	.	.	3.53	134	.
3.35	3.35	52	48	109	3.94	85	.	.	3.27	102	.
5.14	4.67	58	48	120	4.19	111	.	.	3.37	139	.
4.29	3.84	50	49	103	4.00	96	.	.	3.44	112	.
4.37	4.10	55	49	113	3.71	110	.	.	3.16	130	.
4.82	4.31	47	48	97	4.72	91	.	.	3.63	119	.

FRC PP ATS/ERS Corrected	ERV	RV	RV P G/B	RV PP G/B	RV P G/B Corrected	RV PP G/B Corrected	RV P ATS/ERS	RV PP ATS/ERS	RV P ATS/ERS Corrected	RV PP ATS/ERS Corrected	RV/TLC	VC
.	3.67	0.69	2.24	31	.	.	1.81	38	.	.	8	7.92
.	2.21	0.46	1.38	33	.	.	1.39	33	.	.	9	4.94
.	0.67	1.53	1.63	94	.	.	1.49	103	.	.	25	4.71
.	1.45	1.40	1.79	78	.	.	1.57	89	.	.	19	5.97
.	2.36	2.34	2.01	116	.	.	1.68	139	.	.	25	6.86
.	2.50	0.74	1.71	43	.	.	1.53	48	.	.	11	5.71
.	3.05	1.55	1.83	85	.	.	1.61	97	.	.	19	6.56
.	2.63	1.15	1.94	60	.	.	1.70	68	.	.	15	6.50
.	2.02	2.05	1.62	127	.	.	1.57	131	.	.	28	5.35
.	1.98	2.26	2.14	106	.	.	1.78	127	.	.	24	6.97

VC PP	IC	IC P G/B	IC PP G/B	IC P G/B Corrected	IC PP G/B Corrected	IC P ATS/ERS	IC PP ATS/ERS	IC P ATS/ERS Corrected	IC PP ATS/ERS Corrected	DLCO	DLCO PP Burrows	DLCO PP Miller
113	4.12	4.57	90	.	.	5.02	82	.	.	.	.	.
102	2.68	3.22	83	.	.	3.22	83	.	.	.	.	.
89	3.96	3.71	107	.	.	3.78	105	.	.	36.4	104	93
105	4.47	4.06	110	.	.	4.13	108	.	.	40.2	108	101
110	4.46	3.75	119	.	.	4.57	97	.	.	45.7	136	111
104	3.10	3.44	90	.	.	3.96	78	.	.	36.0	112	91
110	3.44	3.53	97	.	.	4.16	83	.	.	43.2	133	108
107	3.81	3.87	99	.	.	4.25	90	.	.	44.9	129	112
103	3.31	2.91	114	.	.	3.50	94	.	.	34.7	125	92
105	4.92	3.92	125	.	.	4.72	104	.	.	.	.	.

DLCO P ATS/ERS	DLCO PP ATS/ERS	DLco/VA	DLco/VA PP Burrows	DLco/VA PP Burrows STPD	DLco/VA PP Miller	DLco/VA PP ATS/ERS	VA	VA %TLC	VA %Pred TLC G/B	IVC	Raw	Raw PP
43	.	.	.	.	.	.	.	.	.	.	1.71	186
32	.	.	.	.	.	.	.	.	.	.	3.21	204
35	102	6.1	140.1	104	104	121	5.9	94.90	83.52	4.6	1.65	127
38	107	5.91	135	100	102	118	6.86	93.05	89.41	5.76	2.59	216
40	114	5.02	117	85	89	101	9.11	99.00	108.30	6.36	1.88	205
37	99	5.28	121	89	90	104	6.83	105.81	92.47	5.62	1.85	166
38	114	5.4	124.9	92	95	108	8.0	98.09	102.99	5.76	1.76	171
38	117	5.93	138	102	106	119	7.56	98.78	96.06	5.97	1.48	137
34	102	4.92	116	86	85	96	7.05	95.23	106.46	5.32	1.66	138
41	.	.	.	.	.	.	.	.	.	.	1.96	218

Gaw	Gaw PP	sRaw	sRaw PP	sGaw	sGaw PP	Pimax	Pemax
0.588	53	9.36	221	0.108	46	.	.
0.312	43	11.04	234	0.091	43	.	.
0.614	74	6.14	136	0.165	75	.	.
0.395	44	9.40	211	0.108	48	.	.
0.537	48	9.91	234	0.102	43	.	.
0.548	58	6.14	140	0.163	72	.	.
0.571	57	9.02	209	0.111	48	.	.
0.677	70	6.36	146	0.158	69	.	.
0.603	68	7.28	164	0.138	61	.	.
0.512	45	9.45	224	0.106	45	.	.

Appendix A.h.: Electronic cigarette experiment, pulmonary function

ID	GRP	STATUS	Code	AGE	HT (cm)	WT (kg)	SEX	SPIRO	FVC	FVC PP NHANES	FVC P Knudson
201	Naïve	Complete	.	20	198.1	107.0	M	Spiro	7.79	111	6.28
202	User	Complete	.	20	166.1	79.0	M	Spiro	4.91	101	4.39
204	User	Complete	.	19	176.0	90.0	M	Spiro	4.97	94	4.90
205	User	Complete	.	19	182.1	100.0	M	Spiro	5.86	103	5.26
208	User	Complete	.	19	190.0	76.4	M	Spiro	6.58	105	5.73
211	Naïve	Complete	.	19	179.1	72.8	M	Spiro	5.48	100	5.08
214	Naïve	Complete	.	20	182.9	76.4	M	Spiro	6.66	112	5.38
215	Naïve	Complete	.	23	184.9	91.4	M	Spiro	6.42	106	5.72
216	User	Complete	.	25	172.0	62.8	M	Spiro	5.45	105	4.99
218	Naïve	Complete	.	22	193.0	83.8	M	Spiro	6.89	104	6.13

FVC PP Knudson	FEV1	FEV1 PP NHANES	FEV1 P Knudson	FEV1 PP Knudson	FEV1/FVC	FEV1/FVC PP Knudson	25-75%	25-75% PP	PEF	PEF PP	FET100%	MVV
124	5.93	103	5.44	109	76.12	88	4.76	83	11.68	98	12.80	196
112	3.70	90	3.78	98	75.36	88	2.95	65	9.83	108	8.36	155
101	4.20	94	4.23	99	84.51	98	4.35	91	10.33	108	6.98	190
111	4.98	105	4.54	110	84.98	98	5.21	104	12.70	126	7.07	201
115	6.09	118	4.95	123	92.55	107	6.49	122	10.12	94	6.73	207
108	4.65	101	4.38	106	84.85	98	5.15	106	10.09	103	9.06	208
124	4.93	100	4.65	106	74.02	86	3.92	76	10.20	97	7.33	174
112	5.27	106	4.94	107	82.09	95	5.50	108	11.96	111	6.92	228
109	4.20	98	4.19	100	77.06	92	3.53	78	9.68	100	9.70	193
112	5.66	104	5.30	107	82.15	95	5.51	101	11.52	100	9.96	223

MVV PP	fb	LUNG VOL	TLC	TLC P G/B	TLC PP G/B	TLC P G/B Corrected	TLC PP G/B Corrected	TLC P ATS/ERS	TLC PP ATS/ERS	TLC P ATS/ERS Corrected	TLC PP ATS/ERS Corrected
85	90	LungVol	8.55	9.16	93	.	.	8.75	98	.	.
95	95	LungVol	5.81	6.15	95	.	.	6.19	94	.	.
103	90	LungVol	6.49	7.09	91	.	.	6.98	93	.	.
102	85	LungVol	7.34	7.67	96	.	.	7.47	98	.	.
97	90	LungVol	9.24	8.41	110	.	.	8.10	114	.	.
109	85	LungVol	6.91	7.38	94	.	.	7.23	96	.	.
88	85	LungVol	8.05	7.72	104	.	.	7.53	107	.	.
114	110	LungVol	7.79	7.87	99	.	.	7.69	101	.	.
112	95	LungVol	7.31	6.62	110	.	.	6.66	110	.	.
103	90	LungVol	9.46	8.65	109	.	.	8.34	113	.	.

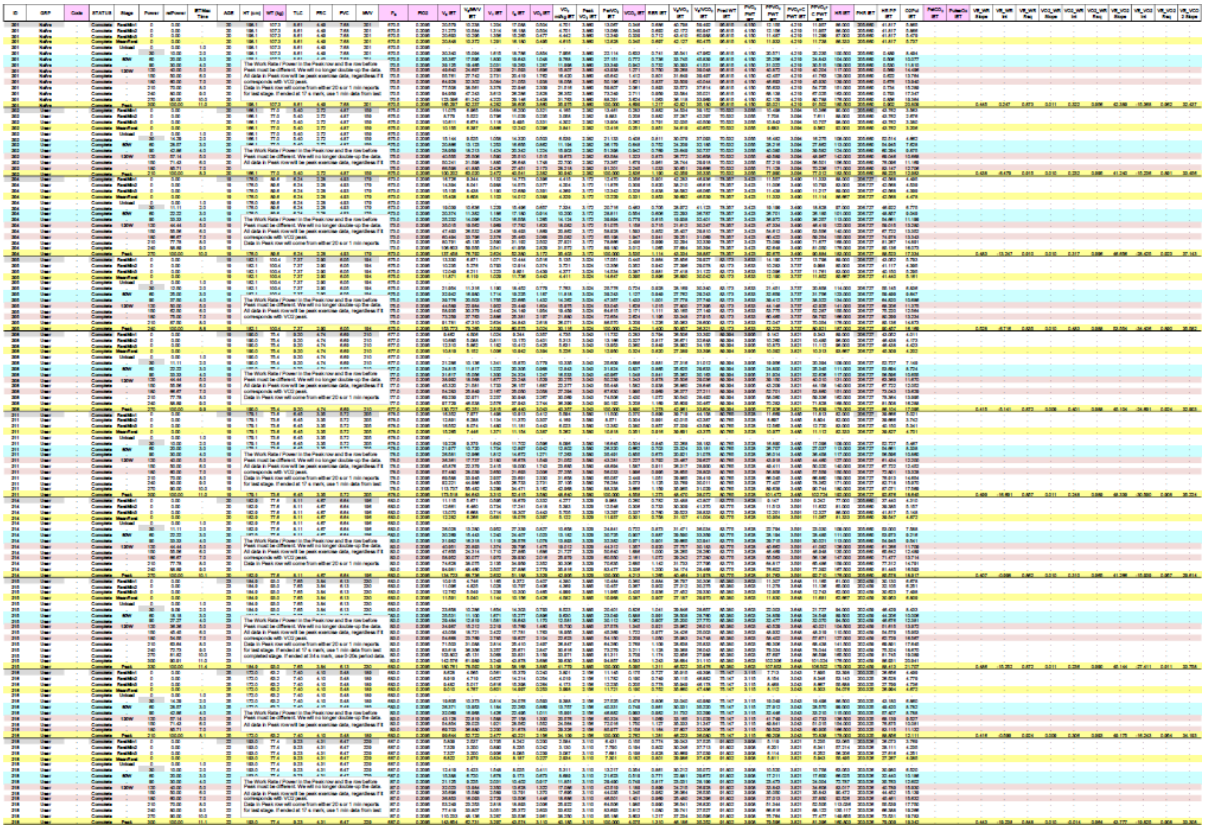
Vtg	FRC PL	FRC %TLC	Pred FRC %TLC	PP FRC %TLC	FRC Pred G/B	FRC PP G/B	FRC P G/B Corrected	FRC PP G/B Corrected	FRC P ATS/ERS	FRC PP ATS/ERS	FRC P ATS/ERS Corrected
4.98	4.66	55	48	114	4.59	102	.	.	3.73	125	.
3.21	2.83	49	48	102	2.92	97	.	.	2.98	95	.
3.62	2.55	39	48	82	3.39	75	.	.	3.20	80	.
3.57	3.11	42	48	89	3.61	86	.	.	3.34	93	.
5.88	5.45	59	48	123	4.68	116	.	.	3.53	155	.
3.48	3.15	46	48	95	3.98	79	.	.	3.27	96	.
5.03	4.58	57	48	119	4.17	110	.	.	3.37	136	.
4.14	3.90	50	49	103	3.99	98	.	.	3.44	113	.
4.38	4.04	55	49	113	3.71	109	.	.	3.16	128	.
5.25	4.45	47	48	97	4.73	94	.	.	3.63	123	.

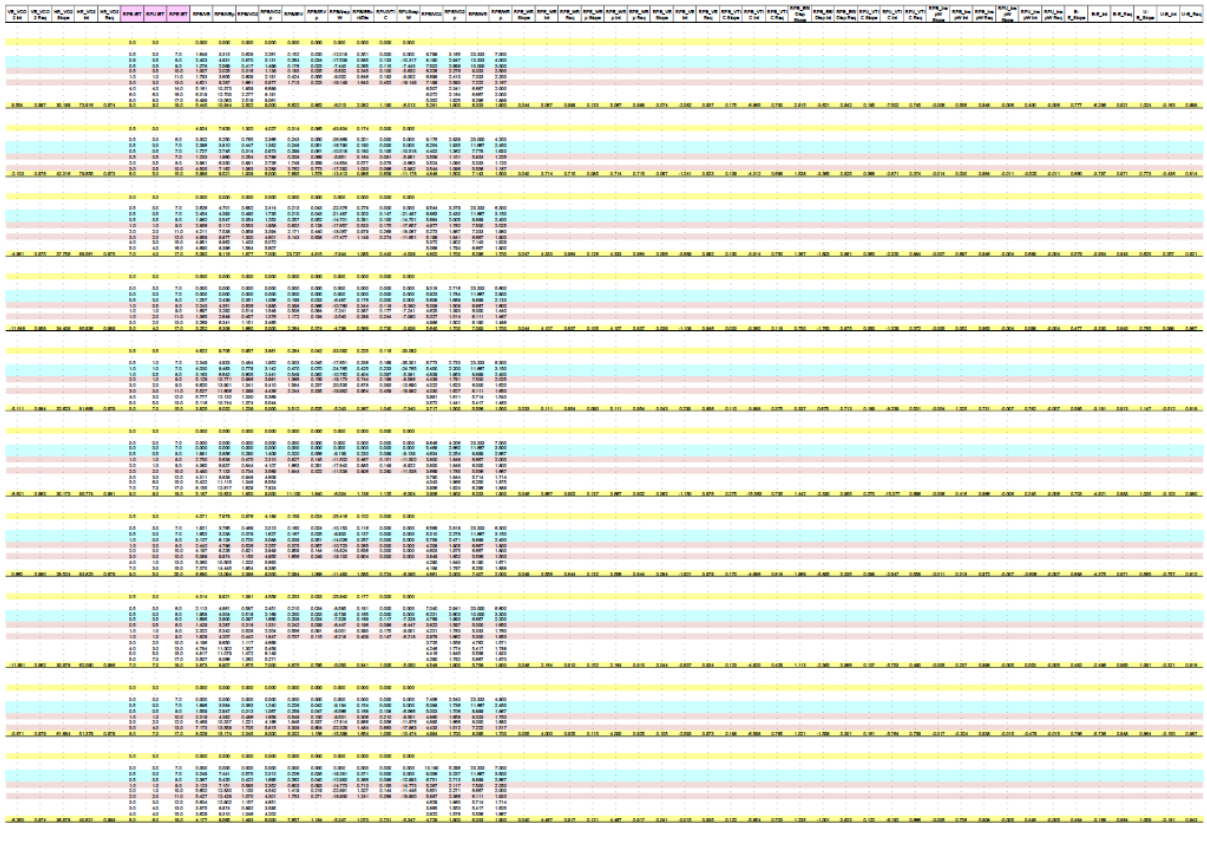
VC PP	IC	IC P G/B	IC PP G/B	IC P G/B Corrected	IC PP G/B Corrected	IC P ATS/ERS	IC PP ATS/ERS	IC P ATS/ERS Corrected	IC PP ATS/ERS Corrected	DLCO	DLCO PP Burrows	DLCO PP Miller
108	3.88	4.57	85	.	.	5.02	77	.	.	.	.	.
110	2.98	3.22	92	.	.	3.22	93	.	.	.	.	.
88	3.94	3.71	106	.	.	3.78	104	.	.	38.7	111	99
104	4.23	4.06	104	.	.	4.13	102	.	.	40.1	108	100
106	3.79	3.73	102	.	.	4.57	83	.	.	47.8	143	116
97	3.76	3.40	111	.	.	3.96	95	.	.	.	.	.
112	3.47	3.55	98	.	.	4.16	83	.	.	42.1	129	105
113	3.89	3.88	100	.	.	4.25	92	.	.	43.6	125	109
104	3.27	2.91	112	.	.	3.50	93	.	.	37.0	133	98
108	5.01	3.92	128	.	.	4.72	106	.	.	.	.	.

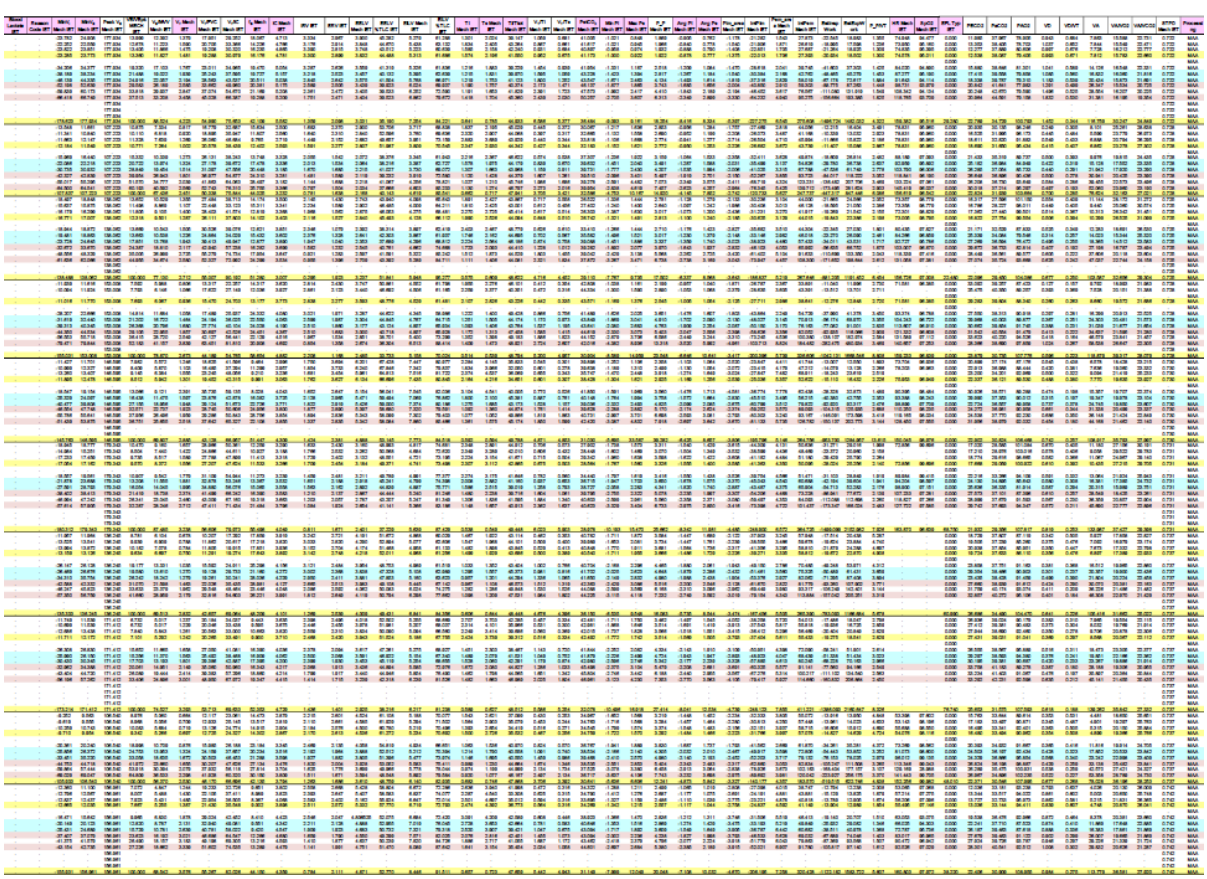
DLCO P ATS/ERS	DLCO PP ATS/ERS	DLco/VA	DLco/VA PP Burrows STPD	DLco/VA PP Burrows STPD	DLco/VA PP Miller	DLco/VA PP ATS/ERS	VA	VA %TLC	VA %Pred TLC G/B	IVC	Raw	Raw PP
43	.	.	.	.	.	.	.	.	.	.	2.04	222
32	.	.	.	.	.	.	.	.	.	.	3.01	192
35	109	6.1	138.9	103	103	120	6.4	98.05	89.65	5.2	1.72	132
38	107	5.91	137	100	102	118	6.79	92.40	88.50	5.70	3.34	278
40	119	5.35	124	90	95	108	8.93	96.68	106.22	5.92	1.94	213
37	.	.	.	.	.	.	.	.	.	.	1.94	178
38	111	5.3	121.4	89	92	105	8.0	99.21	103.45	5.73	1.77	171
38	113	5.82	136	100	104	116	7.48	96.02	95.05	5.94	1.56	144
34	108	5.16	122	90	89	101	7.17	98.02	108.20	5.24	1.56	130
41	.	.	.	.	.	.	.	.	.	.	1.58	177

Gaw	Gaw PP	sRaw	sRaw PP	sGaw	sGaw PP	Pimax	Pemax
0.504	45	10.21	241	0.102	43	.	.
0.337	47	9.53	202	0.105	50	.	.
0.583	70	6.22	138	0.161	73	.	.
0.301	34	11.94	268	0.085	38	.	.
0.520	47	11.37	269	0.088	37	.	.
0.526	55	6.74	154	0.151	66	.	.
0.565	56	8.93	206	0.112	49	.	.
0.641	66	6.47	148	0.155	68	.	.
0.644	72	6.82	154	0.147	65	.	.
0.635	56	8.29	196	0.121	51	.	.

Appendix A.i.: Electronic cigarette placebo, incremental exercise test



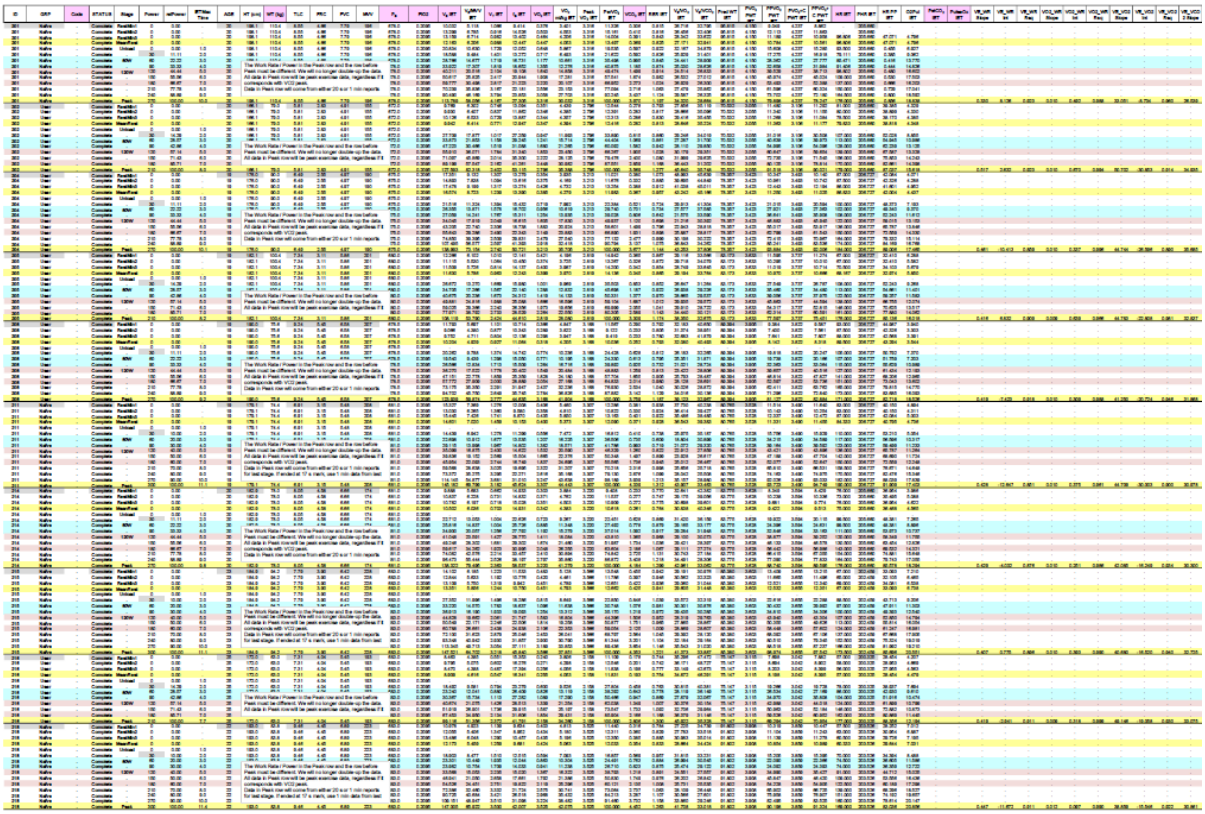


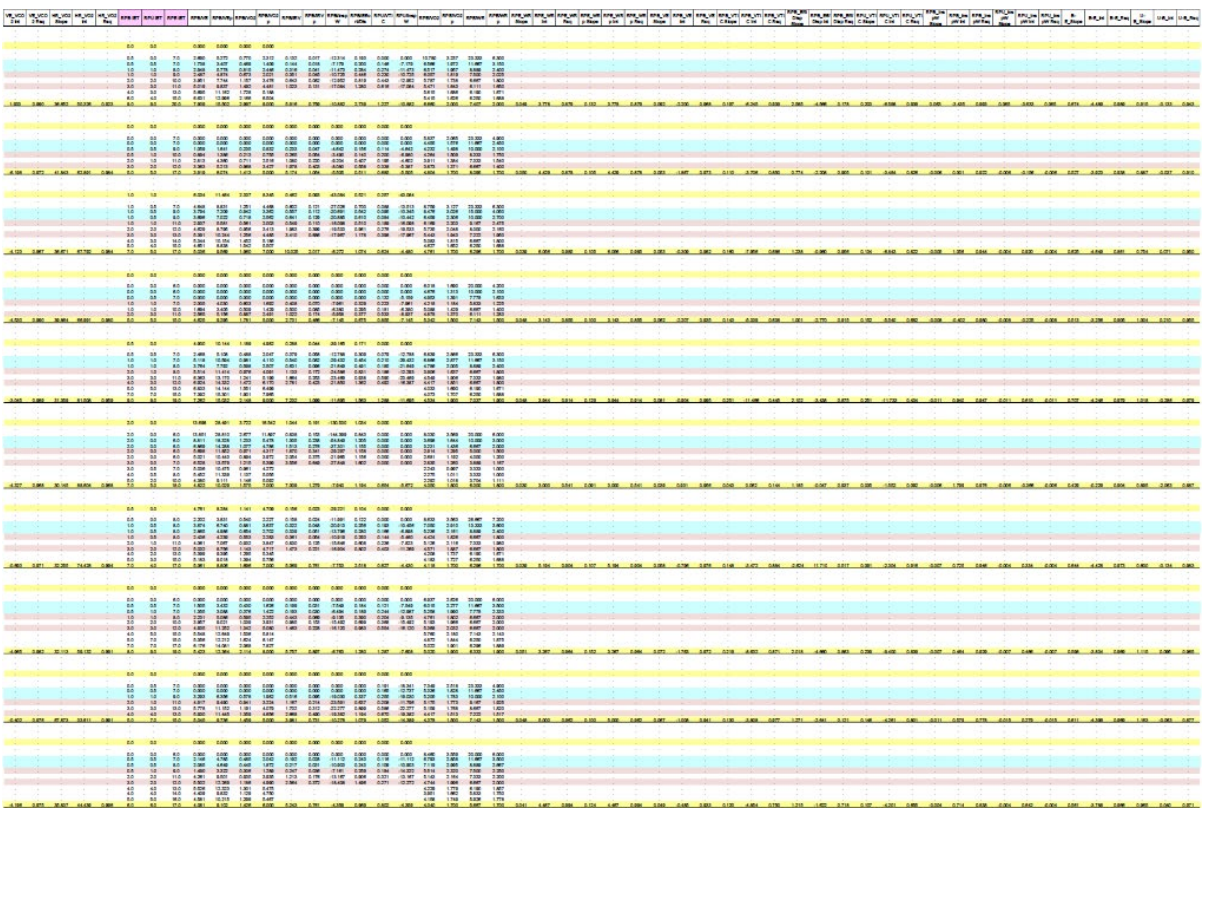


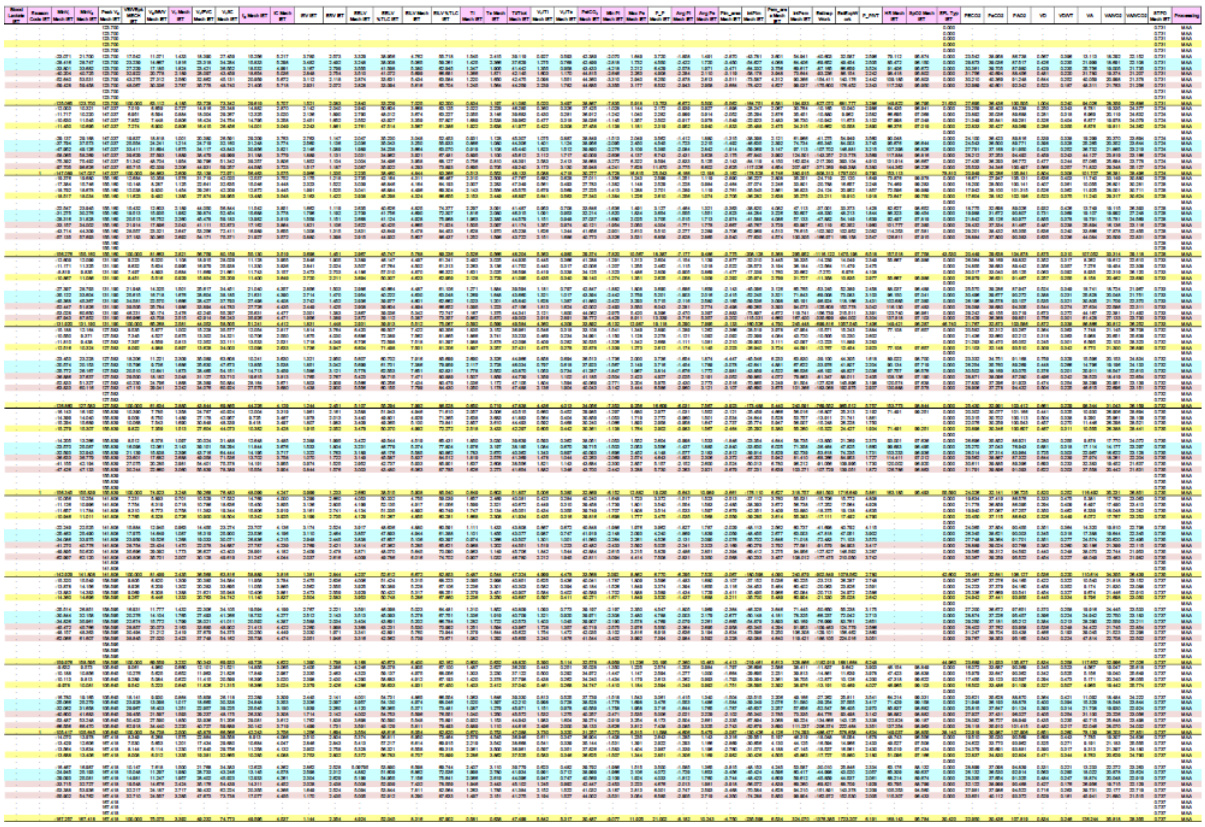
ID	GRP	Status	PeakVO2_Form	PeakVO2_DYS1	PeakVO2_DYS2	PeakVO2_DYS3	PeakVO2_VAS1_UNPLEA	PeakVO2_VAS2a_DEPR	PeakVO2_VAS2b_ANX	PeakVO2_VAS2c_FRUS	PeakVO2_VAS2d_ANGER	PeakVO2_VAS2e_FEAR
201	Naïve	Complete	A	2	7	6	9.7	0.7	7.2	7	7.2	6.8
202	User	Complete	D	8	5	6	7.5	0.1	1.8	7.6	0.3	0.1
204	User	Complete	B	5	11	1	3.7	0	0	1.3	0	0
205	User	Complete	C	7	11	6	8	0	0	6	6.9	0
208	User	Complete	C	5	2	11	7	0.5	0.7	4.3	3.2	1.9
211	Naïve	Complete	A	4	6	1	7.3	0	0	7.7	7.9	0
214	Naïve	Complete	B	5	2	6	5.7	0	0	5.6	0.5	0
215	Naïve	Complete	D	2	11	5	7.9	2.1	3	5.9	5.4	6.9
216	User	Complete	D	1	4	5	7.8	0	0	0.6	0	0.5
218	Naïve	Complete	A	5	11	13	6.1	0	0	0	0	0

	Questionnaire Data Key
1	My breath does not go in all the way.
2	My breathing requires effort.
3	I feel that I am smothering.
4	I feel hunger for air.
5	My breathing is heavy.
6	I feel out of breath.
7	My chest feels tight.
8	My breathing requires work.
9	I feel that I am suffocating.
10	My chest is constricted.
11	I feel that my breathing is rapid.
12	My breathing is shallow.
13	I feel that I am breathing more.
14	I cannot get enough air.
15	My breath does not go out all the way.

Appendix A.j.: Electronic cigarette experiment, incremental exercise test







ID	GRP	Status	PeakVO2_Form	PeakVO2_DYS1	PeakVO2_DYS2	PeakVO2_DYS3	PeakVO2_VAS1_UNPLEA	PeakVO2_VAS2a_DEPR	PeakVO2_VAS2b_ANX	PeakVO2_VAS2c_FRUS	PeakVO2_VAS2d_ANGER	PeakVO2_VAS2e_FEAR
201	Naïve	Complete	A	2	8	6	9.2	1	5	7.5	8.6	0.8
202	User	Complete	C	8	2	11	6.7	0	3.4	7.8	1.1	0
204	User	Complete	D	11	5	15	8.7	0	0	2.1	0	0
205	User	Complete	B	11	5	6	8.8	0	0	6.1	5.6	0
208	User	Complete	C	5	2	13	8.2	0	0.5	1.2	0.7	0
211	Naïve	Complete	B	14	5	6	8.9	0.1	0.1	4.8	5	0.1
214	Naïve	Complete	D	5	11	13	6.9	0	0	4.7	1.8	1.8
215	Naïve	Complete	C	2	11	5	8.6	2	3.6	4.9	4.2	6.3
216	User	Complete	A	1	5	7	7.3	0	2.3	2.5	0	2.1
218	Naïve	Complete	C	4	5	13	6.3	0	0	0.2	0	0

	Questionnaire Data Key
1	My breath does not go in all the way.
2	My breathing requires effort.
3	I feel that I am smothering.
4	I feel hunger for air.
5	My breathing is heavy.
6	I feel out of breath.
7	My chest feels tight.
8	My breathing requires work.
9	I feel that I am suffocating.
10	My chest is constricted.
11	I feel that my breathing is rapid.
12	My breathing is shallow.
13	I feel that I am breathing more.
14	I cannot get enough air.
15	My breath does not go out all the way.

Appendix A.k.: Electronic cigarette placebo smoking challenge

ID	GRP	Status	AGE	HT (cm)	WT (kg)	Sex	#Points ECIG	PkPm ECIG	1sPm ECIG	IntPm ECIG	preSpO2 ECIG	postSpO2 ECIG	preHR ECIG	postHR ECIG
201	**Naïve**	**Complete**	20	198.1	107.3	M	20.0	-18.5	-18.1	-24.7	96.6	96.5	81.0	83.2
202	**User**	**Complete**	20	166.1	77.0	M	20.0	-20.3	-20.1	-29.1	94.4	93.6	57.0	60.9
204	**User**	**Complete**	19	176.0	89.6	M	20.0	-18.7	-18.6	-26.4	97.4	96.8	74.3	79.4
205	**User**	**Complete**	19	182.1	100.4	M	20.0	-25.7	-24.3	-20.2	95.0	94.6	72.4	70.8
208	**User**	**Complete**	19	190.0	75.4	M	20.0	-19.1	-19.0	-29.4	98.6	97.0	65.7	65.6
211	**Naïve**	**Complete**	19	179.1	73.6	M	20.0	-22.1	-21.8	-34.2	98.2	100.0	70.1	73.2
214	**Naïve**	**Complete**	20	182.9	77.6	M	20.0	-21.6	-21.4	-28.6	97.2	96.4	67.6	65.4
215	**Naïve**	**Complete**	23	184.9	93.0	M	20.0	-20.1	-19.7	-27.4	97.9	98.7	57.2	51.6
216	**User**	**Complete**	25	172.0	63.2	M	20.0	-21.8	-21.5	-24.7	99.0	98.7	58.2	56.2
218	**Naïve**	**Complete**	22	193.0	77.4	M	20.0	-21.7	-21.4	-35.7	97.0	95.3	62.5	65.4

Appendix A.l.: Electronic cigarette experiment smoking challenge

ID	GRP	Status	AGE	HT (cm)	WT (kg)	Sex	#Points ECIG	PkPm ECIG	1sPm ECIG	IntPm ECIG	preSpO2 ECIG	postSpO 2 ECIG	preHR ECIG	postHR ECIG
201	**Naïve**	**Complete**	20	198.1	110.4	M	.	.	.	**.**	.	.	.	**.**
202	**User**	**Complete**	20	166.1	79.0	M	.	.	.	**.**	98.7	96.7	63.1	**57.0**
204	**User**	**Complete**	19	176.0	90.0	M	20.0	-20.4	-20.3	**-29.1**	97.6	97.6	67.6	**64.2**
205	**User**	**Complete**	19	182.1	100.4	M	20.0	-26.9	-26.2	**-22.8**	94.1	94.8	75.7	**62.4**
208	**User**	**Complete**	19	190.0	75.6	M	20.0	-19.4	-19.1	**-28.8**	97.2	96.8	66.0	**62.3**
211	**Naïve**	**Complete**	19	179.1	74.4	M	20.0	-20.5	-20.2	**-28.7**	98.9	100.0	67.7	**64.1**
214	**Naïve**	**Complete**	20	182.9	78.0	M	20.0	-20.1	-19.9	**-27.4**	99.1	97.5	69.4	**64.5**
215	**Naïve**	**Complete**	23	184.9	94.2	M	13.0	-12.5	-11.8	**-10.4**	.	.	.	**.**
216	**User**	**Complete**	25	172.0	63.0	M	20.0	-21.6	-20.9	**-18.9**	98.8	97.6	44.2	**46.8**
218	**Naïve**	**Complete**	22	193.0	83.8	M	20.0	-21.3	-21.3	**-38.4**	98.2	97.9	43.7	**45.7**

Appendix B.a.: Informed consent, formaldehyde study

Appalachian State University
Informed Consent for Participants in
Research Projects Involving Human Subjects

Title of Project: Effect of Formaldehyde Exposure on Pulmonary and Vascular Function
IRB Study #:
Principal Investigators (co-PIs): Stephen Ratchford, Ph.D.
Jonathon Stickford, Ph.D.
Ashley Goodman, Ph.D.

Research Assistants: Marc Augenreich Email: augenreichma@appstate.edu
Val Province Email: provincevm@appstate.edu
Sera Denlea Email: denleasc@appstate.edu
Taylor Lovci Email: lovcit@appstate.edu
Kennedy Williams Email: williamska8@appstate.edu
Kyle Kimball Email: kimballkc@appstate.edu

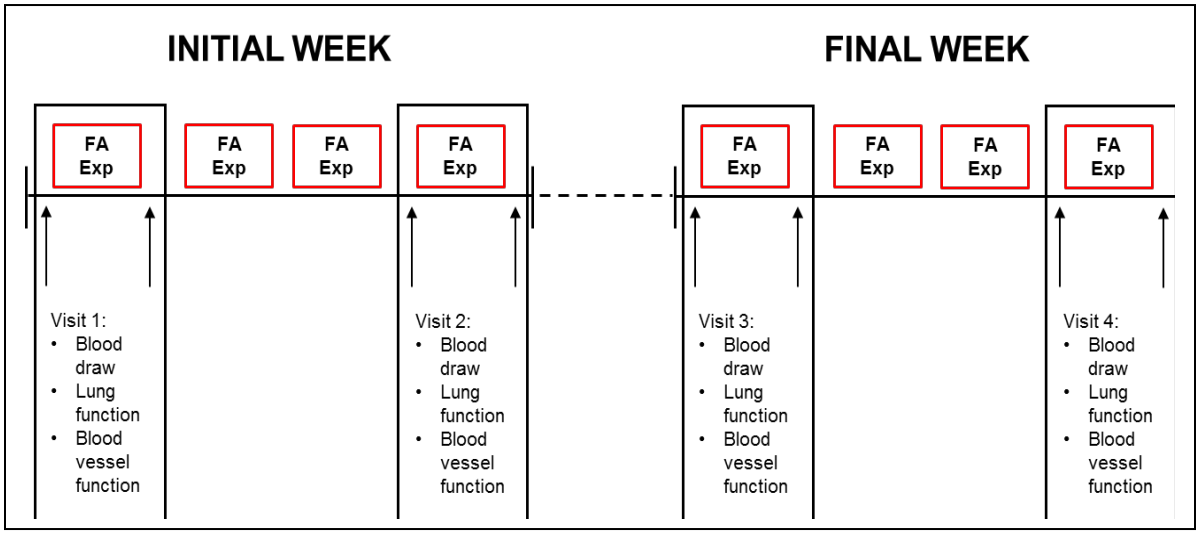
This is to confirm that I,_____, have been given the following information with respect to my participation in a research study under the supervision of Drs. Stephen Ratchford, Jonathon Stickford, and Ashley Goodman, to which Marc Augenreich, Val Province, Taylor Lovci, Sera Denlea, Kennedy Williams, and Kyle Kimball may be assisting.

1. **Purpose of the study:** Dr. Ratchford and his colleagues are conducting a series of research studies to learn more about lung and blood vessel health when individuals come in contact for formaldehyde (FA), a typical chemical used for preserving cadavers in anatomy classrooms.

   The purpose of this work is to better understand lung and blood vessel health and the contributing factors that could cause lung and blood vessel function to become impaired with chemical exposure. The long-term goal of this work is to find optimal strategies to minimize the impact of FA among those with acute and chronic exposure to FA.

2. **Inclusion Criteria: You may participate in the study if the following apply to you:**
   - Over 18 years old
   - Are Healthy
   - Nonsmoker

   Exclusion Criteria: You should not participate in this study if any of the following apply to you:
   - Under 18 years old
   - Relatively unhealthy
   - Smokers
   - Pregnant or those who are trying to become pregnant



3. **Procedures:** Please read the descriptions of each study and experimental visits, writing your initials in the space provided. While you may agree to participate in one or more procedures associated with one or more of the following protocols, you will always have the right to forego one or all procedures associated with this study.

PRELIMINARY SCREENING DAY (0.5-1 hours). A signed consent form will be obtained prior to any research procedures. A screening questionnaire will be filled out that will include questions concerning risks such as: age, family health history, smoking, hypertension, hypercholesterolemia, and physical activity and allergies (iodine, latex, drugs, etc).

Experimental Visits (2 hours/visit, 4 visits total).

Lung and blood vessel function testing will be performed before and after acute and (up to 3) repeated exposures to FA within a week at the start and end of . Typically, acute exposures may occur during the first-class period of a donor-based classroom experience. Repeated exposures may occur over multiple class meetings within the initial week of a donor-based classroom experience. A single visit encompasses lung and blood vessel function testing before and after acute FA exposure. Thus, we are asking you to complete two visits within a single week to perform lung and blood vessel function testing.

Rational for Flexibility in Repeated Exposure Visits. Several types of classroom FA exposure sites are being investigated in this study. Duration and frequency of exposure will vary with each course. For example, Athletic Training Cadaver Dissection at Appalachian State University will meet for 4 hours each day for a time-intensive 5 weeks, while undergraduate anatomy courses may only meet for ~2 hours each week over the course of 16 weeks. Several students will be investigated among a variety of repeated exposures and FA exposure concentrations to gauge the effect of multiple FA exposures. Ideally, these follow-up visits will be at regular intervals throughout the subject's predetermined exposure duration (e.g. once a month for 3 months). Therefore, students may be studied up to 3 different times following an initial exposure which may be as frequent as once a week or as infrequent as once a month based on subject willingness, availability, and investigative team availability for frequent testing to capture repeated FA exposures.

Figure 1. *Example schematic of testing acutely and upon repeated formaldehyde (FA) exposures at Elon University and Appalachian State University where FA concentrations range from 0.04-0.13 ppm. Prior to each day of testing, resting pulmonary and vascular function assessments will be performed on participants as well as immediately following FA exposure. BD, Blood Draw; FA Exp, Formaldehyde Exposure; h, hour; P/V A, Pulmonary/Vascular Assessments; w, week.*

Techniques and Measurements

Blood sampling: A venous blood sample (up to 24ml) will be drawn from the arm on two occasions during each visit (before and after FA exposure). Venous blood will be collected using standard techniques while at rest to test for markers of inflammation and oxidative stress.

Lung Function Testing. Lung function will be tested noninvasively in accordance with the standards set by The American Thoracic Society and The European Respiratory Society. The test will measure the volume of air contained within the lungs, as well as the speed at which you can empty your lungs. You will be asked to perform the tests in a seated, upright position while wearing nose clips. Measurements will include: forced vital capacity (FVC; the amount of air exhaled forcefully and quickly after inhaling as much as you can), forced expiratory volume in one second (FEV1; the amount of air expired during the first second of the FVC test), peak expiratory flow (PEF; the fastest rate that you can force air out of your lungs), and forced expiratory flow (FEF; the rate of air flow during the middle half of the FVC test). These measurements are accepted as a general tool for respiratory health and provide an indication of how well the lungs are working. These tests will typically take 15-20 minutes.

Blood Vessel Function Testing. Blood vessel function will be measured at rest while laying down. Blood vessel function tests may include flow mediated dilation (FMD) and passive limb movement (PLM), two clinically relevant tests to investigate vascular function and nitric oxide bioavailability-- a potent vasodilatory pathway in blood vessels which decreases with chronic inflammation, reactive oxygen species, and cardiovascular disease. Additionally, pulse wave velocity (PWV) may be assessed to determine any alterations to arterial stiffness, another clinically relevant test which has been well documented in healthy, aged, and clinical populations and which may allow for cross comparison to other human models of inflammation-related vascular dysfunction. Dr. Ratchford has extensive experience in this area and has mobile equipment specifically for the purpose of field-based experiments such as those proposed here.
These tests will typically take 30 minutes.

Repeated exposures (**up to 3 follow up visits**) may occur within the same week as the acute exposure or may take place throughout the semester. These additional visits may be decided based on preliminary findings, subject availability and willingness to participate in ongoing, longitudinal data tracking, and investigative team availability and logistical constraints with coordinating between two university settings.

Exercise: Described exercise modalities are small muscle mass and therefore only minimally tax the heart and lungs, even at maximal efforts. All exercise testing may be completed in Leon Levine Hall or a convenient meeting location (place of residence, clinical office, or other private assessment facility) under investigator supervision.

Flow-mediated vasodilation (FMD) test: A blood pressure cuff will be placed on the upper arm and inflated to >250 mmHg for five minutes to partially occlude the forearm. Ultrasound Doppler measurements will be made in the brachial artery to evaluate FMD upon cuff release. Dr. Ratchford has direct experience with this technique (1).

Heart Rate: A standard ECG will be used to measure heart electrical activity, with heart rate determined from R-R interval (lead II).

Limb Blood Flow: An ultrasound Doppler system (Logiq e, GE Medical Systems, Milwaukee, Wisconsin, USA) equipped with two linear array transducers operating at an imaging frequency of 7-8 MHz and 12-14 MHz will be used for the leg and arm, respectively. Vessel diameter will be determined at a perpendicular angle along the central axis of the scanned area, where the best spatial resolution is achieved. The brachial artery (BA) of the right arm will be insonated approximately midway between the antecubital and axillary regions, medial to the biceps brachii muscle. The blood velocity profile will be obtained simultaneously using the same transducers with a Doppler frequency of 4.0-5.0 MHz, operated in the high-pulsed repetition frequency mode (2-25 kHz) with a sample depth of 1.5-3.5 cm and the probe maintained at an insonation angle of 60° or less. Sample volume will be maximized according to vessel size and centered, verified by real-time ultrasound visualization of the vessel. Using artery diameter and V_{mean}, blood flow is calculated as: blood flow (ml/min) = $V_{mean} \cdot \pi \cdot (\text{Vessel Diameter}/2)^2 \cdot 60$. At all sample points, arterial diameter and angle-corrected, time-averaged, and intensity-weighted mean blood velocity (V_{mean}) values can be calculated using commercially available software (Logiq e). Ultrasound digital images and velocity spectra segments of five minutes will be recorded and saved to the GE Logiq e hard drive for off-line image and waveform analysis. Dr. Ratchford has extensive experience with this methodology.

Non-invasive arterial blood pressure: Systemic arterial blood pressure (ABP) will be measured non-invasively on a continuous basis using finger photoplethysmography (Finometer, Ohmeda, Madison, WI, USA). The finometer cuff is placed on the middle finger of the right hand and is supported on a modified surgical stand adjusted to position the finger cuff at heart level.

Passive Leg Movement: Participants are moved into an upright sitting position for ~10 min before the start of the data collection and remain in this position throughout the entire protocol. The protocol consists of 60-s of resting baseline data acquisition followed by a 2-min bout of passive leg extension. PLM is achieved by a member of the research team moving the participant's lower leg through a range of motion, defined by 90° and 180° knee joint angles, at a rate of 1 Hz. Throughout the protocol, the non-moving leg remains fully extended and supported. Real-time feedback to the investigator is provided by a metronome to maintain the cadence. Before commencing, and throughout the protocol, participants are encouraged to remain passive and resist any urge to assist with leg movement. To avoid a startle reflex and active resistance to the PLM, participants are made aware that the assessment will start in the next minute, but to minimize the chance of an anticipatory response, they are not informed of exactly when movement will begin (2). There are no known risks of this procedure.

Pulse-Wave Velocity (PWV) Test: Participants with be in the supine position in a quiet room for ~10 min before the start of data collection. An ascending aortic pressure waveform is derived from the carotid artery waveforms using applanation tonometry with a high-fidelity micromanometer. The carotid-femoral aortic PWV may be determined using the Sphygmocor XCEL system by sequentially recording electrocardiographic-gated carotid and femoral artery waveforms. Distances from the carotid sampling site to the suprasternal notch and from the

suprasternal notch to the femoral artery site are measured as straight lines between the points on the body surface using a tape measure. The time (t) between the onset of femoral and carotid waveforms is determined as the mean from 10 consecutive cardiac cycles. The PWV is calculated from the distance between measurement points (D) and the measured time delay (t) as follows: PWV = D/t (m/sec), where D is distance in meters and t is the time interval in seconds.

Dr. Ratchford has extensive experience in all described procedures, and he or a qualified/trained member of the research team will perform all necessary procedures to ensure safety and validity of assessments.

4. Risks and Discomforts:

All of the research methodology has been conducted on hundreds of subjects in sessions similar to the study proposed.

Blood sampling: There is a possibility of bruising from blood draws. It should be noted that all blood collection procedures will be performed in a clean environment by qualified personnel (i.e., nurse or phlebotomist). The safety of the participant is of utmost importance during the blood draws, therefore standard precautions will be used including the cleaning of the venipuncture site with alcohol, the use of new sterile disposable needles/syringes and changing of disposable gloves in between participants.

Lung function testing: Risks associated with lung function testing are minimal. There is a possibility of discomfort while performing the forced exhalation maneuver.

Exercise: There is a very small risk that performing exercise reveals a problem with your heart (exposing you to the risk of a heart attack or irregular heartbeat that could require hospitalization), and particularly with the blood vessels which supply the heart (coronary arteries). These problems could range from insufficient blood flow to the heart (myocardial ischemia), heart attack (myocardial infarction), or irregular heart beat (arrhythmia), and these serious heart conditions could be fatal. Symptoms for these conditions would be pain in the chest, excessive shortness of breath, or abnormalities on the electrocardiogram (ECG) during exercise. This procedure (the ECG) is performed to be as sure as possible that there are no active heart problems during the study. However, if these problems develop, exercise will be stopped immediately and you may be referred to your local physician for proper follow-up. Finally, the performance of exercise may also result in muscle soreness that might last several days.

Flow-mediated vasodilation (FMD) test: Minor discomfort may be associated with the five-minute occlusion period caused by the blood pressure cuff. However, trained research personnel will make sure that the blood pressure cuff is completely deflated as soon as the measurement is completed.

Handgrip Exercise: Small muscle mass exercise minimizes chances of increasing heart rate and blood pressure, but it may still increase these by ~20bpm or ~40mmHg, respectively. For this reason, these measurements are monitored continuously and the test will be terminated if systolic blood pressure increases above 220 mmHg. There is also a small chance of hand discomfort when isometrically squeezing the hand dynamometer. If the hand grip exercise

becomes too uncomfortable, the subject has the ability to withdraw from the exercise at any time.

Handgrip Exercise MVCs: There is a small chance of hand discomfort when isometrically squeezing the hand dynamometer. If the hand grip exercise becomes too uncomfortable, the subject has the ability to withdraw from the exercise at any time.

Limb Blood Flow: Doppler ultrasound is used to assess limb blood flow. This is a non-invasive assessment and does not pose any known risk other than minor discomfort from moving the ultrasound probe over the skin. Skin tears rarely occur while moving a doppler ultrasound probe over sensitive/fragile skin regions (e.g. morbidly obese individuals with skin folds) and is no more common than what a subject with this type of skin would normally experience in daily life.

Near Infrared Spectroscopy (NIRS): There is no known risk associated with using this device for it is intended use.

Non-invasive arterial blood pressure: There may be minor discomfort during the measurement of arm blood pressure and during the cuff occlusion due to the inflation of the blood pressure cuff.

Passive Leg Movement: Individuals with knee pain or joint impairments may experience mild discomfort during the movement through 90-degree range of motion of the knee joint. Also, individuals with peripheral edema or peripheral neuropathy may experience some discomfort while the ankle is help in place by a member of the investigative team who is assigned to be the leg mover. For this reason, these individuals will be excluded from this test.

Pulse-Wave Velocity (PWV) Test: Apart from the possibility of some minor discomfort associated with the slight pressure that is applied over the artery, there are no significant risks associated with this test.

Reproductive Risks: While this study is strictly characterizing FA exposure, exposure to FA may harm an unborn child. Pregnant women must not take part in this study, nor should women who plan to become pregnant during the study. Women who are at risk of pregnancy will be asked to have a pregnancy test before taking part to exclude the possibility of pregnancy. If you could become pregnant you must use an effective contraceptive during the course of this study. Acceptable methods of birth control include oral/topical/injected contraceptives, intra-uterine devices, and barrier methods. If you become pregnant while taking part in the study, you must immediately tell your research doctor, where options will be discussed with you at that time. Whether or not you remain on study treatment, we will follow the outcome of your pregnancy and we will continue to follow you according to the study plan.

5. Benefits:

a. To You: We will be able to share any data collected on you during the study. However, we will not be able to interpret any clinical results we obtain from you. While this data may be helpful in assessing your physical condition, members of the investigative team do not have the right to make clinical conclusions regarding your health status as it pertains to the above-mentioned investigations.

b. To Society: This study will provide helpful information to those exposed to various levels and durations of FA which can be helpful for understanding the impact on lung and blood vessel health among the general population.

6. **Alternative procedures that could be utilized:** There are not any alterative procedures. The procedures outlined in this document are the only interventions and assessments the investigative team will ask of you. The procedures used in this study are frequently used in research and are the most appropriate methods to accomplish the goals of this research.

7. **Statement of confidentiality:** Volunteers are coded using the first two letters of your first name and first two letters of your last name for statistical analyses. All records are kept in a secure, physically-locked and/or password-protected location, and only the study investigators will have access to the data. All records associated with your participation in the study will be subject to the university confidentiality standards and in the event of any publication resulting from the research no personally identifiable information will be disclosed. Your information will be combined with information from other people taking part in the study. When we write up the study to share it with other researchers as well as the scientific and medical community, we will write about the combined information. You will not be identified in any published or presented materials. To ensure that your information is kept confidential, identification codes but not names will be used on all documents. The Office of Human Research Protections in the U.S. Department of Health and Human Services, the U.S. Food and Drug Administration (FDA), the Office for Research Protections at Appalachian State University and the Institutional Review Board may review records related to this project.

8. **Right to ask questions:** Stephen Ratchford, Ph.D. (828-262-7630) or RatchfordSM@AppState.edu with questions, complaints, or concerns about this research. If you have any questions about your rights as a research subject, please contact the IRB Administrator at the Appalachian State University Institutional Review Board Office at (828) 262-2692, irb@appstate.edu.

9. **Compensation for your time:**

You will be compensated at a rate of $20 for each visit. For completion of all mentioned 4 visits, you will receive $80 after the study is completed or prorated based on participation.

10. **Injury Clause:** In the unlikely event you become injured as a result of your participation in this study, standard emergency procedures will be followed. If you get hurt or sick when you are not at the research site, you should call your doctor or call 911 in an emergency. If your illness or injury could be related to the research, tell the doctors or emergency room staff about the research study, the name of the Principal Investigator (Stephen Ratchford), and provide a copy of this consent form if possible. Please call the PI (Stephen Ratchford, Ph.D. 828-262-7630). You will be responsible for any costs for medical care not paid by your insurance company. No other compensation is offered by Appalachian State University. By signing this document, you are not waiving any legal rights that you have against Appalachian State University for injury resulting from negligence of the University or its investigators.

11. **Voluntary participation:** Your participation in this study is strictly voluntary and will not affect your relationship with the study team, work, academic, or clinical staff. You may withdraw from this study *at any time* by informing the research personnel. You may decline to answer certain questions and may decide not to comply with certain

procedures. However, your being in the study may be contingent upon answering these questions or complying with the procedures. The researcher may end your role in the study without your consent if the researcher deems that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document. You have been given an opportunity to ask any questions you may have, and all such questions or inquiries have been answered to your satisfaction.

Informed Consent Signatures

You must be 18 years of age or older to take part in this research study. If you agree to take part in this research study and have read the information outlined above, please sign your name and indicate the date below. You will be given a copy of this signed and dated consent form for your records.

Participant, Printed Name	**Participant, Signature**
Date	

I, the undersigned, have defined and explained the studies involved to the above volunteer.

Consent Obtainer, Printed Name	**Consent Obtainer, Signature**
Date	

Appendix B.b.: Informed consent, electronic cigarette study

Appalachian State University
Informed Consent for Participants in
Research Projects Involving Human Subjects

Title of Project: **Respiratory limitations, exercise tolerance, and exertional dyspnea following acute electronic cigarette use**
IRB Study #: **16-0297**

Principal
Investigator: Jonathon Stickford, Ph.D. Email: stickfordjl@appstate.edu

Research Assistants: Erica Larson, B.S. Email: larsone@appstate.edu
Jayvaughn Oliver, B.S. Email: oliverjt@appstate.edu
Rylie Bragg Email: braggre@appstate.edu

This is to certify that I, _____ have been given the following information with respect to my participation as a volunteer in a program of investigation under the supervision of Jonathon Stickford, Ph.D. to which Erica Larson, B.S, Jayvaughn Oliver, B.S., and Rylie Bragg may be assisting.

1. **Purpose of the study:**

   Electronic cigarettes (EC) are devices intended to deliver an aerosol containing nicotine and possible flavorings. They are marketed as a method for quitting tobacco smoking, as a healthier alternative to tobacco smoking, and as a method to "smoke" in public spaces. Many teenagers and young adults are increasingly using the devices. Yet, these devices may alter breathing responses exhibited during exercise. Furthermore, the perception of breathlessness during exercise may be affected following EC usage.
   The primary objective of this study is to investigate the acute effects of EC use on breathing responses during exercise and the perception of breathlessness in young adults. The results of this study will help us to understand the health effects of acute EC use in young adults.

2. **Inclusion Criteria: You may participate in the study if the following apply to you:**
   - Sex: Only males will be included in the study. Females will not be included due to the influence of the menstrual cycle on breathing responses.
   - Ethnicity: Any
   - Age: 18 – 25 years of age. The rationale for the cut-off point for the upper limit of this age range is that we wish most of our subjects to come from the college community, whereas older students may present different physiological and psychological responses than that of younger students.
   - Interest in participating in a research study investigating electronic cigarette use
   - Understand written and oral instructions in English
   - Provide informed consent
   - Available during times the data collection is offered.

- According to the American College of Sports Medicine (ACSM) exercise preparticipation recommendations, does not have known cardiovascular, metabolic, or renal disease, nor exhibits any signs or symptoms of cardiovascular, metabolic, or renal disease.
- Lung function must be within a specific range (FVC $\geq$ 80% of predicted, 2) $FEV_1 \geq 80\%$ of predicted, and 3) $FEV_1/FVC \times 100 \geq 75\%$)
- You must not be a tobacco cigarette smoker.
- You must be either a non-tobacco cigarette smoker (i.e., you can be an EC user) or naïve to EC. However, if you currently use EC, you must abstain from EC usage in the 12 hours prior to each laboratory visit.
- You must participate in regular vigorous conditioning exercise such as running, jogging, aerobics, cycling, or swimming, less than two times per week to be included. You can be included if you participate in daily, unorganized physical activity.

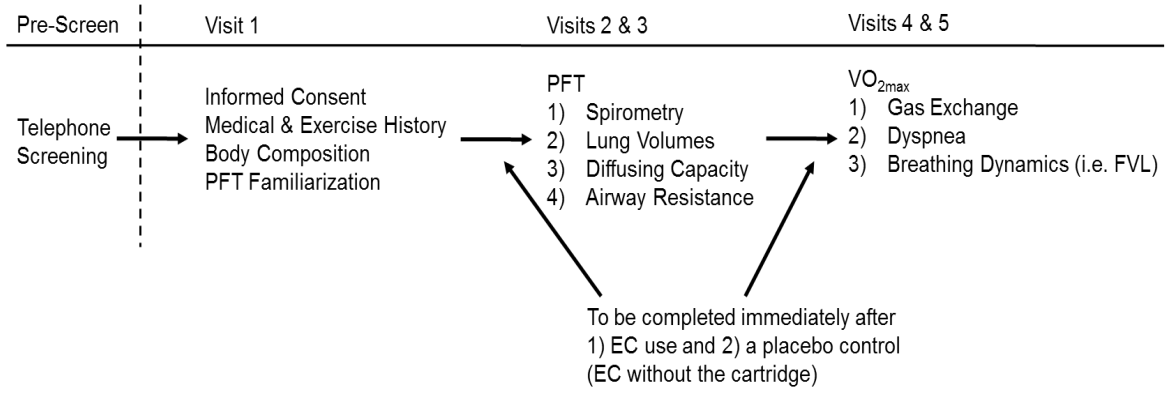
Exclusion Criteria (i.e., a list of criteria that if any one applies to you would prohibit you from being included in this study):

- Known cardiovascular, metabolic or renal disease, or signs/symptoms suggestive of cardiovascular, metabolic or renal disease will exclude you from participation. Individuals with these characteristics require medical clearance before exercise participation, according to the 2015 ACSM preparticipation health screening recommendations.
- If lung function is outside of a specific range, you will be excluded.
- Current tobacco cigarette smoker.
- If you participate in regular vigorous conditioning exercise such as running, jogging, aerobics, cycling, or swimming, two times or more per week, you will be excluded. Men who participate in daily, unorganized physical activity, will not be excluded.

3. **Procedures:**. ***Please read the descriptions of each experimental day and write your initials in the space provided.***

You could be asked to repeat a trial, procedure, or test. This could happen for many reasons such as equipment failure, power outage, inconclusive test results, etc. However, you do not have to repeat a trial, procedure, and/or test if you do not wish to do so.

Below is a timeline showing all visits and experiments which you will complete in this study.



_____ initial **Prescreen:** You may be telephoned by the Principle Investigator or a Research Assistant (see page 1) and asked screening questions to determine your eligibility for the study.

Visit 1:

_____ initial **Consent and Questionnaires:** Potential participants who meet inclusion criteria will be invited to a screening interview within the laboratory located off campus (Charleston Forge Research Site). At this screening visit, the study will be explained in-depth to you by the PI or a trained research assistant. You will be provided time to consider your options and get all questions answered - if you agree to participate, you will then provide your written informed consent.

After you have provided consent (~30 minutes), you will be asked to complete questionnaires: 1) a medical history questionnaire (~15 minutes) and 2) an exercise history questionnaire (~15 minutes).

_____ initial **Body Composition:** Following completion of the questionnaires, we will measure your height, weight, waist circumference, and body composition. Your percent body fat will be measured using a Bod Pod plethysmograph. You will sit in a chamber and may hear some clicking while air pressure changes to estimate your body volume. This piece of equipment estimates your body's composition of fat and muscle by air movement. This is accomplished by measuring your mass (scale) and body volume (relationship between pressure and volume in chamber) and entering it into a calculation for fat mass and fat free mass (muscle). This is an extremely accurate method and presents no risk. These procedures will take ~15 minutes total

_____ initial **Pulmonary Function Testing (PFT) Familiarization:** You will be asked to become familiar with tests of breathing function; the protocol will follow that described by the American Thoracic Society. These tests include measurement of the total volume of air your lungs can hold, the volume of air that you can push out with one maximal breath, the volume of air that you can forcefully breathe out in one second, and the maximum volume of air that you can breathe in 12 seconds. For all these procedures, you will wear nose clips and breathe through a disposable mouthpiece. These procedures will take ~30 minutes total.

_____ initial **Activity Assessment:** You will be asked to wear an accelerometer around your waist over the 7 days following Visit 1in order to monitor your physical activity level. You will return the accelerometer during your next visit.

Visits 2 and 3:

_____ initial **EC usage:** You will be asked to inspire from an EC during each of these two visits. On one occasion the EC will contain a cartridge containing nicotine and flavoring. On the other occasion, the EC will not contain any nicotine or flavoring to serve as a control. You will inspire from the EC device once every 30 seconds for 10 minutes. A display on a computer screen will provide you with information on when to inspire from the EC. You will be asked to expire the vapor into a bag, which will be emptied following the testing session, to eliminate secondhand "smoke" exposure to yourself and study personnel. This will take ~15 minutes total.

_____ initial **Pulmonary Function Testing (PFT):** You will be asked to perform tests of breathing function following EC use. The protocol will follow that described by the American

Thoracic Society. These tests include measurement of the total volume of air your lungs can hold, the volume of air that you can push out with one maximal breath, the volume of air that you can forcefully breathe out in one second, and the maximum volume of air that you can breathe in 12 seconds. For all these procedures, you will wear nose clips and breathe through a disposable mouthpiece. These procedures will take ~60 minutes total.

Visits 4 and 5:

_____ initial **EC Usage:** You will be asked to inspire from an EC during each of these two visits. On one occasion the EC will contain a cartridge containing nicotine and flavoring. On the other occasion, the EC will not contain any nicotine or flavoring to serve as a control. You will inspire from the EC device once every 30 seconds for 10 minutes. A display on a computer screen will provide you with information on when to inspire from the EC. You will be asked to expire the vapor into a bag, which will be emptied following the testing session, to eliminate secondhand "smoke" exposure to yourself and study personnel. This will take ~15 minutes total.

_____ initial **Maximal Aerobic Capacity Exercise Test ($\dot{V}O_{2max}$):** You will be asked to perform a maximal exercise test following EC use. This test will measure your highest exercise capacity and is often described as a $\dot{V}O_{2max}$ test. You should be rested, well nourished, and hydrated for the test and avoid alcohol 12 hours before the test. Additionally, avoid caffeine and tobacco 3 hours before the test. Avoid significant exertion or exercise the day of testing and report any medication that you are using to the testing staff before the test. When you are ready to perform the test, the investigators will help with necessary adjustments to testing equipment to assure your comfort. You will be fitted with a rubber mouthpiece and nose clip. This procedure will require ~45 minutes total, with exercise lasting approximately 15 minutes.

Cycling Protocol
You will perform cycling exercise on a stationary bicycle. Prior to exercise, you will rest sitting on the bike with both hands on the handle bars for 5 minutes. After this rest period you will warm up at a light intensity of 30 Watts (W) using a cadence of 60 RPM for 5 minutes. The work rate will then be increased by 30W every minute until volitional exhaustion or signs/symptoms prohibit further exercise testing. During each minute of the test, we will measure your physiological responses (e.g., heart rate, VO_2, etc.) and perceptual responses (e.g., ratings of perceived breathlessness, unpleasantness of breathlessness, and perceived exertion). You will be asked to "rate your breathing" by pointing with your finger to a number on a scale (Borg 0-10), which will represent your perceived level of breathlessness. The number will be repeated out loud in order to confirm your choice. During the exercise you may have an even stronger or greater intensity of breathlessness than you have previously experienced. If this occurs, you should point to the word "maximal" if the severity is greater than 10. After the mouthpiece is removed, you can tell us the number. Following completion of the test, you will perform a brief light intensity cool-down.

Flow-Volume Loops
During the maximal exercise test, the speed at which you breathe air in and out and the volume of air you breathe will be measured. Approximately once every 60 seconds during the exercise test, you will be prompted to breathe in completely, filling your lungs with air, and then return to normal breathing. Before and after the exercise test, while at rest, you will be prompted to perform 3 breathing maneuvers, where you complete a maximal inhalation (filling your lungs completely with air) followed by a complete exhalation (breathe out all the air you can). The

investigators will coach you through these maneuvers. These procedures are performed during the maximal exercise test, and add no additional time.

_____ initial **Breathlessness Questionnaire**: Following the exercise test, you will be asked to complete a questionnaire about the breathing sensations that you experienced during exercise. During this "debriefing session" we will give you a questionnaire including 15 respiratory sensation descriptors to describe the respiratory sensations you were most often experiencing (e.g., respiratory work/effort, air hunger, and/or chest tightness).

3. Discomforts and risks:

There are minimal risks involved with measuring/monitoring/performing: questionnaires, physical characteristics, body composition, activity assessment, pulmonary function testing, flow-volume loops, and breathlessness during exercise.

EC Usage: The risks of EC use are not clear, in part, because 1) they are not regulated by the US Food and Drug Administration, and 2) the chemical composition varies with each brand and flavoring. In other words, in addition to nicotine, there will be other chemicals in the EC that re not disclosed by the manufacturers. However, acute usage may lead to minor throat irritation, cough, and dry mouth. Side effects of nicotine use may include light-headedness, nausea, and addiction risk. Long-term risks of EC are unknown.

Maximal Aerobic Capacity Exercise Test (VO_{2max}): VO_{2max} test risks include abnormal heart beats, abnormal blood pressure responses, muscle cramps, muscle strain and/or joint injury, delayed muscle soreness (1 to 2 days afterwards), light headedness, fatigue, and in rare instances, heart attack.

Loss of Confidentiality: Any time information is collected; there is a potential risk for loss of confidentiality. Every effort will be made to keep your information confidential; however, this cannot be guaranteed.

Other Risks: There may possibly be other side effects that are unknown at this time. If you are concerned about other, unknown side effects, please discuss this with the researchers.

How you can help reduce some of the risks: During your participation in this research, the researchers will closely observe your testing to determine whether there are problems that need medical care. It is your responsibility to do the following:

- Ask questions about anything you do not understand.
- Keep appointments.
- Follow the study researchers' instructions.
- Let the researchers know if your telephone number changes.
- Tell the researchers before you take any new medication.
- Tell your regular doctor about your participation in this research.
- Talk to a family member or friend about your participation in this research.

4. a. Benefits to me: You can expect to receive knowledge of your cardiovascular conditioning and physical fitness. You will receive a copy of your lung function data. These screenings are

being performed solely for research purposes and are not to be construed as a clinical screening intended for diagnostic or therapeutic purposes. The screening data will not be read for any health care or diagnostic purpose. Under no circumstance will the investigator, research staff or other University employee interpret your screening as normal or abnormal and they are unable to make any medical comments or interpretations based on your results. Please contact your health care provider if you have any questions regarding this data.

b. Potential benefits to society: The results of the study will aid in our understanding of the acute health effects attributed to EC and will serve as a foundation to study the long-term effects of EC on overall health in young adults, which are research areas recently identified by the National Institutes of Health. Furthermore, findings from the study could increase physician awareness of the effects of EC on breathlessness during exercise and may alter their diagnostic strategy.

5. **Alternative procedures that could be utilized:** Not participating in the study.
   The procedures used in this study are frequently used in research and are the most appropriate methods to accomplish the goals of this research.

6. Time duration of the procedures and study:

   _____ initial Pre-screening (about 20-30 min).

   You will need to visit the Charleston Forge Laboratory for the following:

   _____ initial Visit 1 (about 1.75 hours).

   _____ initial Visit 2 (about 1.25 hours).

   _____ initial Visit 3 (about 1.25 hours).

   _____ initial Visit 4 (about 1 hour).

   _____ initial Visit 5 (about 1 hour).

   Approximately 6.75 hours Total

7. **Statement of confidentiality:** Volunteers are coded by an identification number for statistical analyses. All records are kept in a secure location. All records associated with your participation in the study will be subject to the university confidentiality standards and in the event of any publication resulting from the research no personally identifiable information will be disclosed. The Office of Human Research Protections in the U.S. Department of Health and Human Services, the U.S. Food and Drug Administration (FDA), the Office for Research Protections at Appalachian State University and the Institutional Review Board may review records related to this project.

8. **Right to ask questions:** Please contact Jonathon Stickford, Ph.D. (828-262-7471), with questions, complaints, or concerns about this research. If you have any questions about your rights as a research subject, please contact the IRB Administrator at the Appalachian State University Institutional Review Board Office at (828) 262-2692, irb@appstate.edu. This study has been approved on 8/1/16 by the Institutional Review Board (IRB) at

Appalachian State University. This approval will expire on 8/2/17 unless the IRB renews the approval of this research.

9. **Compensation:** You will receive a total of $50 upon completion of this study:
Compensation Breakdown: You will receive $10 per experimental protocol completed (each of Visits 2-5). Additionally, you will receive $10 upon completion of the study. Total: $50
You may be asked to repeat a trial. If you agree to repeat a trial, you will be paid for the repeated trial as stated above.

   NOTE: Current University policy requires the collection of Social Security numbers (or Appalachian Banner ID numbers) if study compensation is more than $100 for a single study or $599 for participation in multiple studies in a calendar year. ***Since the compensation for this study is not more than $100, you will not need to provide your address and Social Security number (or Appalachian Banner ID number) when you complete the form for payment.***
   The University is required to annually report these payments to the IRS. This may require you to claim the compensation that you receive for participation in this study as taxable income.

10. **Injury Clause:** In the unlikely event you become injured as a result of your participation in this study, standard emergency procedures will be followed. If you get hurt or sick when you are not at the research site, you should call your doctor or call 911 in an emergency. If your illness or injury could be related to the research, tell the doctors or emergency room staff about the research study, the name of the Principal Investigator, and provide a copy of this consent form if possible. Please call the PI as soon as possible (Jonathon Stickford, Ph.D. 828-262-7471). You will be responsible for any costs for medical care not paid by your insurance company. No other compensation is offered by Appalachian State University. By signing this document, you are not waiving any legal rights that you have against Appalachian State University for injury resulting from negligence of the University or its investigators.

11. **Voluntary participation:** Your participation in this study is voluntary. You may withdraw from this study at any time by informing the research personnel. You may decline to answer certain questions and may decide not to comply with certain procedures. However, your being in the study may be contingent upon answering these questions or complying with the procedures. The researcher may end your role in the study without your consent if the researcher deems that your health or behavior adversely affects the study or increases risks to you beyond those approved by the Institutional Review Board and agreed upon by you in this document. You have been given an opportunity to ask any questions you may have, and all such questions or inquiries have been answered to your satisfaction.

    You must be 18 years of age or older to take part in this research study. If you agree to take part in this research study and have read the information outlined above, please sign your name and indicate the date below. You will be given a copy of this signed and dated consent form for your records.

_____

Volunteer Date

I, the undersigned, have defined and explained the studies involved to the above volunteer.

_____

Person Obtaining Consent Date

Appendix C.: Telephone screening form

Initial Telephone Screening Form for Study E-CIG

General Information:

Name: _____

Age: _____ **Sex (only males): _____**

Race: _____
Phone: _____ Email: _____

Address (if no email):_____
Height: _____ Weight: _____ Allergies (latex?):_____

Exclusion Criteria: any question answered "yes" in this section will disqualify the potential subject.

	Yes	No	
1.	☐	☐	Age – outside the ages of 18 and 25 yr?
2.	☐	☐	Do you currently smoke tobacco cigarettes?
3.	☐	☐	Have you ever been diagnosed with a sleep disorder or use CPAP?
4.	☐	☐	Do you have a history of asthma, COPD, or any lung issues?
5.	☐	☐	Do you have a history of an irregular heartbeat or any heart condition? (Have you had an EKG performed?)
6.	☐	☐	Do you have any known health conditions? High blood pressure? Diabetes? Thyroid issues?
7.	☐	☐	Do you regularly exercise more than 2 times per week with a specific health goal in mind?

Inclusion Criteria:

	Yes	No	
1.	☐	☐	Body Mass Index of <30?
2.	☐	☐	Are you able to ride a stationary bicycle?
3.	☐	☐	Do you currently smoke electronic cigarettes?
4.	☐	☐	If you don't currently smoke electronic cigarettes, are you willing to smoke electronic cigarettes while participating in the study?

Appendix D.: Health history form

Appalachian State University – Integrative Human Physiology Laboratories

251 Industrial Park Dr. Boone, NC 28607 Phone:(828)262-7471

ASU **Medical History Form** **Page 1**

Subject ID#:	**Study:**

Age:

Highest Education Achieved:

Ethnicity:

☐ ***Hispanic or Latino.*** A person of Cuban, Mexican, Puerto Rican, South or Central American, or other Spanish culture or origin, regardless of race. The term "Spanish origin" can be used in addition to "Hispanic or Latino."

☐ ***Not Hispanic or Latino.***

Race: What race do you consider yourself to be?

☐ ***American Indian or Alaska Native.*** A person having origins in any of the original peoples of North, South, or Central America, and who maintains a tribal affiliation or community attachment.

☐ ***Asian.*** A person having origins in any of the original peoples of the Far East, Southeast Asia, or the Indian subcontinent, including, for example, Cambodia, China, India, Japan, Korea, Malaysia, Pakistan, the Philippine Islands, Thailand, and Vietnam. (Note: Individuals from the Phillippine Islands have been recorded as Pacific Islanders in previous data collection strategies.)

☐ ***Black or African American.*** A person having origins in any of the black racial groups of Africa. Terms such as "Haitian" or "Negro" can be used in addition to "Black" or "African American".

☐ ***Native Hawaiian or Pacific Islander.*** A person having origins in any of the original peoples of Hawaii, Guam, Samoa, or other Pacific islands.

☐ ***White.*** A person having origins in any of the original peoples of Europe, the Middle East, or North Africa.

☐ ***Check here if you do not wish to disclose any or all of the above information.***

Medications: include over the counter drugs/oral contraceptives/dietary supplements

Name/Dosage/How often taken:

Allergies:

Smoking History:

Do you smoke? Yes No Cigarettes? Pipe / Cigar? Other? If you quit, what year did you quit _____

_____# packs per day for_____# of years What year did you start smoking? _____

Have you ever been exposed to second hand smoke?_____Home_____Work_____Other_____Years

Alcohol Consumption History:

Do you currently drink alcohol? If you drank alcohol previously, when did you stop?

If you ever did drink alcohol, what is (was) the volume consumed?
_____# ounces / day for_____# of years

ASU		Medical History Form	Page 2
Medical History:			
NO	**YES**	**Please explain any "YES" answers below:**	
		high blood pressure	
		swelling	
		chest pain / history of heart attack	
		extra heart beats, racing or fluttering	
		abnormal electrocardiogram (ECG)	
		other heart trouble (e.g. murmur, valve problems)	
		high cholesterol	
		diabetes (e.g. frequent urination and abnormal thirst)	
		seizures	
		stroke	
		fainting or black-out spells, dizziness	
		anxiety (diagnosed)	
		depression (diagnosed)	
		recurrent fatigue (e.g. feeling tired or extreme lack of energy)	
		insomnia or poor sleeping	
		thyroid problems	
		difficulty breathing	
		emphysema/ asthma/ chronic bronchitis	
		cough, sputum (phlegm)	
		tuberculosis	
		chronic infection	
		stomach/GI problems (e.g. heart burn, nausea, vomiting, diarrhea, constipation, abdominal pain, gas pain, black stools, blood in stools)	
		hepatitis	
		bleeding disorder (e.g. bleeding or bruising easily)	
		kidney/ urinary problems (e.g. frequent urination, burning when urinating, urine changing in color)	
		joint injuries/ joint pain, back pain, or leg pain	
		arthritis (rheumatoid or osteoarthritis)	
		hearing problems (e.g. impaired hearing or ringing in the ears)	
		migraine headaches	
		vision problems (exclude corrected near/far sightedness)	
		surgical procedures (e.g. c-sections, appendectomy, augmentations, knee and back surgeries, tonscillectomy, etc)	
Additional Notes:			

ASU	Medical History Form	Page 3
Exercise History:		
Do you currently exercise aerobically?	How many years?	Duration:
	Types of Exercise:	Frequency:
Do you compete in endurance events?	How many years?	Frequency:
	What events?	Athlete in college? Yes No
Any other types of exercise?	How many years?	Duration:
	Types of Exercise:	Frequency:
If you are currently sedentary, when did you last exercise?	How many years?	Duration:
	Types of Exercise:	Frequency:
Weight History:		
If overweight, how long have you been overweight?	Were you overweight as a child?	By how much?
	How many times has your weight changed?	
Any events that led up to your obesity? (E.g. Pregnancy, injury) Yes No	If yes, how may events? 1 2 3 4 5 >5	
Sleep History:		
Have you ever been diagnosed with a sleep disorder?	Yes	No
Do you use CPAP/BIPAP at night?	Yes	No
Do you snore at night?	Yes	No
Has someone ever told you that you snore at night?	Yes	No
Do you have daytime sleepiness?	Yes	No
Women Only:		
Menstrual history: Age begin_____	Regular? ☐ Yes ☐ No	
☐ Heavy ☐ Medium ☐ Light		
If your periods are irregular or associated with excessive bleeding or unusual discharge please elaborate:		
Number of days between periods:_____days	Usual duration of period:_____days	
At what age did menopause occur, if applicable?		
Number of pregnancies?_____Number of births?_____Explain any complications with pregnancy: _____		

Authorization to Release Information - Please check all that applies and sign/date.	
	I authorize Appalachian State University to collect and save the above protected health information on me for purposes of research. I understand that all information is private and confidential.
	I authorize Appalachian State University to keep this information and any information gained from my participation in their studies in a database so that they may contact me.
	The above information is correct and complete to the best of my knowledge.

Signature **Date**

Appendix E.: International physical activity questionnaire

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE (August 2002)

SHORT LAST 7 DAYS SELF-ADMINISTERED FORMAT

FOR USE WITH YOUNG AND MIDDLE-AGED ADULTS (15-69 years)

The International Physical Activity Questionnaires (IPAQ) comprises a set of 4 questionnaires. Long (5 activity domains asked independently) and short (4 generic items) versions for use by either telephone or self-administered methods are available. The purpose of the questionnaires is to provide common instruments that can be used to obtain internationally comparable data on health–related physical activity.

Background on IPAQ

The development of an international measure for physical activity commenced in Geneva in 1998 and was followed by extensive reliability and validity testing undertaken across 12 countries (14 sites) during 2000. The final results suggest that these measures have acceptable measurement properties for use in many settings and in different languages, and are suitable for national population-based prevalence studies of participation in physical activity.

Using IPAQ

Use of the IPAQ instruments for monitoring and research purposes is encouraged. It is recommended that no changes be made to the order or wording of the questions as this will affect the psychometric properties of the instruments.

Translation from English and Cultural Adaptation

Translation from English is supported to facilitate worldwide use of IPAQ. Information on the availability of IPAQ in different languages can be obtained at www.ipaq.ki.se. If a new translation is undertaken we highly recommend using the prescribed back translation methods available on the IPAQ website. If possible please consider making your translated version of IPAQ available to others by contributing it to the IPAQ website. Further details on translation and cultural adaptation can be downloaded from the website.

Further Developments of IPAQ

International collaboration on IPAQ is on-going and an ***International Physical Activity Prevalence Study*** is in progress. For further information see the IPAQ website.

More Information

More detailed information on the IPAQ process and the research methods used in the development of IPAQ instruments is available at www.ipaq.ki.se and Booth, M.L. (2000). *Assessment of Physical Activity: An International Perspective*. Research Quarterly for Exercise and Sport, 71 (2): s114-20. Other scientific publications and presentations on the use of IPAQ are summarized on the website.

INTERNATIONAL PHYSICAL ACTIVITY QUESTIONNAIRE

We are interested in finding out about the kinds of physical activities that people do as part of their everyday lives. The questions will ask you about the time you spent being physically active in the **last 7 days**. Please answer each question even if you do not consider yourself to be an active person. Please think about the activities you do at work, as part of your house and yard work, to get from place to place, and in your spare time for recreation, exercise or sport.

Think about all the **vigorous** activities that you did in the **last 7 days**. **Vigorous** physical activities refer to activities that take hard physical effort and make you breathe much harder than normal. Think *only* about those physical activities that you did for at least 10 minutes at a time.

1. During the **last 7 days**, on how many days did you do **vigorous** physical activities like heavy lifting, digging, aerobics, or fast bicycling?

   _____ **days per week**

   ☐ No vigorous physical activities → ***Skip to question 3***

2. How much time did you usually spend doing **vigorous** physical activities on one of those days?

   _____ **hours per day**

   _____ **minutes per day**

   ☐ Don't know/Not sure

Think about all the **moderate** activities that you did in the **last 7 days**. **Moderate** activities refer to activities that take moderate physical effort and make you breathe somewhat harder than normal. Think only about those physical activities that you did for at least 10 minutes at a time.

3. During the **last 7 days**, on how many days did you do **moderate** physical activities like carrying light loads, bicycling at a regular pace, or doubles tennis? Do not include walking.

   _____ **days per week**

   ☐ No moderate physical activities → ***Skip to question 5***

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

4. How much time did you usually spend doing **moderate** physical activities on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

Think about the time you spent **walking** in the **last 7 days**. This includes at work and at home, walking to travel from place to place, and any other walking that you have done solely for recreation, sport, exercise, or leisure.

5. During the **last 7 days**, on how many days did you **walk** for at least 10 minutes at a time?

_____ **days per week**

☐ No walking → ***Skip to question 7***

6. How much time did you usually spend **walking** on one of those days?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

The last question is about the time you spent **sitting** on weekdays during the **last 7 days**. Include time spent at work, at home, while doing course work and during leisure time. This may include time spent sitting at a desk, visiting friends, reading, or sitting or lying down to watch television.

7. During the **last 7 days**, how much time did you spend **sitting** on a **week day**?

_____ **hours per day**

_____ **minutes per day**

☐ Don't know/Not sure

This is the end of the questionnaire, thank you for participating.

SHORT LAST 7 DAYS SELF-ADMINISTERED version of the IPAQ. Revised August 2002.

Appendix F.: 24-hour health history questionnaire

24-HOUR HEALTH HISTORY

Study: _____ **Age:** _____ **Height:** _____ **Weight:** _____ **Sex:** _____

Subject Number: _____ **Date:** _____

Do you have:	**Yes**	**No**	**How do you feel?**	**# of hours sleep**_____	**# of hours since eating:**_____ **What did you eat?**
Head cold	☐	☐			
Nasal Congestion	☐	☐	Good	**How was your sleep?**	
Headache	☐	☐			
Sore Throat	☐	☐	Fair	Normal	
Digestive Upset	☐	☐			
Intestinal Disorder	☐	☐	Not so good	Wakeful	
General Fatigue	☐	☐			
Muscle Soreness	☐	☐	Bad	Restless	

Medicine taken in last 24 hours:	**Any leg cramps Since last activity?**	**Physical activity in last 24 hours:**	**Any unusual physical activity in last 24 hours?**
_____	**Yes** ☐ **No** ☐	_____	_____

** **Take weight with each visit.**

Appendix G.a.: Perceptual questionnaire, rating of perceived breathlessness

Rate Your Breathing

0 Nothing at all

0.5 Very, very weak (Just Noticeable)

1 Very Weak

2 Weak (Light)

3 Moderate

4 Somewhat Strong

5 Strong (Heavy)

6

7 Very Strong

8

9

10 Very, very strong (Almost max)
Maximal

Appendix G.b.: Perceptual questionnaire, rating of unpleasantness of breathing

Rate Unpleasantness of your Breathing

0 Not Unpleasant

0.5 Very, very weak (Just Noticeable)

1 Very Weak Unpleasantness

2 Weak (Light) Unpleasantness

3 Moderate Unpleasantness

4 Somewhat Strong Unpleasantness

5 Strong (Heavy) Unpleasantness

6

7 Very Unpleasant

8

9

10 Maximal Imaginable Unpleasantness

Appendix G.c.: Perceptual questionnaire, rating of perceived exertion

Rate Your Exercise

6

7 Very, very light

8

9 Very light

10

11 Fairly light

12

13 Somewhat hard

14

15 Hard

16

17 Very hard

18

19 Very, very hard

20

Vita

Marc Andrew Augenreich was born in Syracuse, New York, to Scott and Kelly Augenreich. He graduated from William Amos Hough High School in North Carolina in June 2015. The following autumn, he entered Appalachian State University to study Exercise Science, and in December 2018 he was awarded the Bachelor of Science degree. In the fall of 2019, he accepted a research assistantship in Exercise Science at Appalachian State University and began study toward a Master of Science degree.

Mr. Augenreich has accepted the Molecular Life Sciences Fellowship at the University of Missouri, Columbia where he will pursue a PhD in Nutrition and Exercise Physiology. He currently resides in Blowing Rock, North Carolina with his dog Milla.